Experimental Hemorrhagic Septicemia of Calves with *Pasteurella multocida* Serotype E:2: Clinical, Pathologic and Microbiologic Studies

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Summary

Hemorrhagic septicemia (HS) was experimentally induced in seven-month-old calves (n = 2) by intratracheal inoculation of 10^{10} colony-forming units of six-hour log-phase *Pasteurella multocida* serotype E:2 to study its clinicopathologic features and microbiology. The incubation period was within four hours postinfection. The general continuum of clinical signs in order of manifestation was pyrexia, anorexia, dyspnea, swelling of the throat-forelimb region, tympany, nasal discharge, profuse salivation, lethargy, recumbency and death. The prominent lesions observed at necropsy were congestion of the lungs with consolidation and pleural adhesions of the apical lobes, pleurisy, edematous swelling of the throat and dewlap which exuded yellowish serum-like fluid, petechial and ecchymotic hemorrhages. Histologically, the lung lesions were typical of fibrinous bronchopneumonia with thickened alveolar septa, hyperemia, edema and cellular responses of the lungs. *P. multocida* E:2 was re-isolated bacteriologically from the lungs, lymph nodes, liver, kidneys, spleen, edema fluid, and heart blood at necropsy. The organism was not detected in the venous blood until a few hours before death. The clinical and pathological features seen in the animals showed that there were striking similarities with *P. multocida* type B:2 HS. The data should help veterinarians recognize suspected cases of HS in the field.

■ INTRODUCTION

Hemorrhagic septicemia (HS) is an acute and often fatal disease principally occurring in cattle and water buffaloes, but occasionally other domesticated and wild mammals can be affected. The disease occurs almost exclusively in Asia and Africa, although outbreaks have been reported in Europe and North America (4, 15). HS usually occurs as a primary pasteurellosis, but latent infections such as trypanosomosis have reportedly precipitated clinical HS (5). *Pasteurella multocida* serotypes B:2 (6:B) and E:2 (6:E) are the principal causes of HS. Although serotype B:2 has been mainly reported in Asian countries and E:2 in African countries (4, 5, 9), both serotypes have been recovered from the disease in some African

countries (11). Besides type B:2, several other B serotypes (B:3, B:4 and B:3,4) have been incriminated in recent years in sporadic outbreaks of HS in cattle and feral ruminants such as deer, elk and bison (15).

Unlike bovine HS due to *P. multocida* B:2, very little is known about the pathogenesis of bovine HS caused by *P. multocida* E:2. In Africa, serotype E:2 appears to be dominant, with cattle rather than water buffaloes being mainly involved. The extent of losses from the disease are represented by mortalities of 200 to 10,000 cattle reported in Zambia and Zimbabwe (6, 10). Published literature on HS in Africa is limited to epidemiological studies. While the literature abounds on the pathogenesis of *P. multocida* type B:2, reports on the clinicopathologic features of type E:2 are fragmentary (12). Although definitive diagnosis of HS is currently made by bacteriological identification of the causative agent, the veterinarian in the field may rely on clinical and pathological features to tentatively diagnose the disease. The paucity of information on the clinical and pathological features of HS in Africa has made

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the diagnosis of the disease in the field difficult. This is further complicated by the fact that other diseases such as anthrax, rinderpest and clostridial disease are analogous to HS in the field (4). There are thus more speculations than accurate diagnosis of HS since the disease occurs mainly in regions where husbandry practices are primitive, and consequently disease surveillance and diagnostic systems are poorly developed.

This preliminary study was designed to reproduce and highlight the clinicopathologic signs and the microbiology of HS caused by *P. multocida* E:2 in susceptible calves, and thus to assist veterinarians in recognizing cases in the field.

■ MATERIALS AND METHODS

Experimental animals

Six- to seven-month-old conventionally reared susceptible calves in apparently healthy conditions and free of *P. multocida* were used. In preinoculation sera, antibody levels to *P. multocida* type E:2, as determined by the indirect hemagglutination (IHA) (3) test, were negative. Prior to infection of the calves, vital signs (rectal temperature, pulse and respiratory rates) were monitored daily for one week to observe whether they remained afebrile and free of any clinical signs of diseases.

Infection method

The calves were dosed intratracheally with 10 ml bovine isolate of *P. multocida* serotype E:2 grown with slow shaking (150 rpm) at 37ºC for 6 hours in Lab-Lemco broth (Oxoid) enriched with 0.5% yeast extract. The dose represented 10^{10} colony forming unit or $1000 LD_{50}$ (1), while the control calves were inoculated intratracheally with 10 ml of sterile enriched Lab-Lemco broth.

Experimental design

Four calves were allocated to two groups of two calves and housed in separate rooms of the same building. One group was inoculated with *P. multocida* serotype E:2 while the other group served as a sham-inoculated control. Clinical scores were recorded daily after infection and the calves that died were necropsied.

Clinical observations

The calves were observed twice daily after inoculation at which time rectal temperature and clinical signs were recorded until death occurred.

Microbiology

Nasal swabs for isolation of bacteria were collected before inoculation and daily after inoculation until death of the animals. The swabs were cultured on blood agar, incubated overnight at 37ºC. Tissues or organs obtained at necropsy were also cultured for bacteria on blood agar. The isolates were confirmed as *P. multocida* E:2 by the IHA procedure (3) and gel diffusion precipitin test (7).

Necropsy procedure

At death the calves were necropsied and gross lesions recorded. Control calves were euthanatized and necropsied at the end of the period of observation (120 h postinoculation). Tissues were fixed in 10% phosphate buffered formalin for 24 h before embedding in paraffin for sectioning. Sections were cut at 4 to 6 microns and all were stained with hematoxylin and eosin and examined microscopically.

■ RESULTS

Clinical observations

The incubation period observed via the intratracheal route of infection lasted in this study about four hours. The clinical course of the disease in the calves lasted from about four hours up to 120 hours (five days) postinfection. During these time intervals, the prominent signs included increased rectal temperature, depression, anorexia, respiratory distress, profuse salivation, recumbence and death (Table I). Edematous swelling was observed in the throat, dewlap and around the forelimbs regions. No clinical signs were observed in the control.

Necropsy findings

At postmortem, subcutaneous edema with serogelatinous fluid in the submandibular, throat and dewlap region were the most conspicuous lesions. The subcutaneous tissue was strewn with petechial hemorrhages. The cases were diagnosed as fibrinous bronchopneumonia, with little fibrin exudation, some interstitial lymph vessel thrombosis, and features of suppurative bronchitis. While the lungs of one of the calves had generalized congestion, the second calf had in addition consolidation of the right apical lobe, pleurisy, pericarditis and adhesion of the right lungs to the coastal wall. Lobulated appearance of the lungs due to thickening of the interlobular septa was evident. Cross sections of consolidated lobes showed mostly dark red lobules, with small grey-white foci of alveolar size in place of air filled spaces. Fibrin in interstitial septa was not prominent. Bronchi contained copious amounts of purulent pasty material, especially obvious when pressure was applied to the adjacent parenchyma.

On low power microscopic examination, the lesions resembled those of a multifocal inflammatory process; few or many alveoli near bronchi and bronchioles were filled with inflammatory exudates and there was partial necrosis of the bronchial mucosa. Fibrin in alveoli was less prominent; inflammatory cells in alveoli were a mixture of macrophages and neutrophils. On higher magnification, bacterial colonies were seen in most lobules. There was vascular thrombosis, especially of capillaries. Lobular necrosis did not occur. The prescapular, bronchial, hepatic and mesenteric lymph nodes were enlarged and incision of these nodes revealed a gelatinous fluid. The liver and bile ducts were inflamed, but the spleen and kidney were normal in appearance. The internal lining of the gastrointestinal tract was dark and showed signs of hyperemia with hemorrhages. Necroses of the muscle fibers and vasculitis were observed. The prescapular lymph nodes had fibrin and edema in the nodal tissues with accompanying thrombosis of blood and lymphatic vessels. There were no gross or microscopic changes in the control calves.

Bacteriological re-isolation

Pasteurella multocida was not isolated from the nasal passages of the calves prior to challenge. The *P. multocida* E:2 inoculum was re-isolated from the challenged animals from a variety of samples, including nasal secretions at the later stages of infection, 2-3 days postinfection. Edema of the throat-dewlap-forelimb was rich in *P. multocida* at the different stages of the clinical course. At necropsy, *P. multocida* was isolated from the lung, kidney, liver, spleen, heart blood and lymph nodes. Cultures of blood were negative until about the 60th hour postinfection. *Pasteurella* isolation from bone marrow was positive about 24 hours after the animals' death. *P. multocida* was not isolated from blood or any other tissues of the control calves.

Table I

Synopsis of the clinical course of hemorrhagic septicemia observed in calves
following intratracheal challenge with *Pasteurella multocida* serotype E:2

Time (hours)	Mean temperature (°C)	Mean respiratory rate/min	Mean pulse rate/min	Other clinical signs
0	38.5	24	82	Animals look normal
4	40.2	28	88	Rough hair coat, reduced appetite, dyspnea
12	41.0	27	90	Listlessness, anorexia, mucus nasal discharge, ocular discharge, dyspnea
18	39.5	26	112	Anorexia, dyspnea, tympany, edema of dewlap and throat regions, lethargy
24	41.0	27	94	Mucus nasal discharge, profuse salivation, anorexia, increased edema of dewlap, reduction in skin turgor (dehydration), lethargy
30	41.8	34	80	Mucus nasal discharge, drooling, dyspnea, anorexia, edema of throat and dewlap, lethargy, recumbence
36	39.8	24	74	Mucus nasal discharge, dyspnea, edema of throat-forelimb region, anorexia, lethargy, recumbence (and death of one of the calves)
48	40.5	24	84	Surviving calf shows mucus nasal discharge, reduced dyspnea, edema, anorexia, lethargy
54	40.9	26	80	Same as at 48th hour
60	41.0	24	70	Mucus nasal discharge, anorexia, edema of throat-forelimb region, lethargy
72	39.8	26	84	Mucus nasal discharge, slight appetite, dyspnea, edema, tremor
78	40.5	22	104	Mucus nasal discharge, edema of dewlap, tympany, dehydration, emaciation, lethargy
84	40.5	20	108	Mucus nasal discharge, dullness, reduced appetite, diarrhea, lethargy, recumbence
96	38.8	22	80	Diarrhea, dyspnea, foamy salivation, lethargy, recumbence
102	39.9	20	72	Same as at 96th hour
120	38.8	18	70	Death of second calf

■ DISCUSSION

In the natural HS disease, the *P. multocida* organism is believed to gain entry via the tonsillar region of the nasopharynx following inhalation or ingestion (1, 5). In the natural disease the frequently reported signs are either the acute form of the disease with sudden death of the calves with no apparent clinical signs, or the subacute form characterized by pneumonic traits of HS. Although there are no clear-cut differences, Rhoades et al. (14) observed that in experimentally induced HS, the nature of the lesions depended on the route of infection; pneumonic lesions dominated when the route of infection was intranasal. The intratracheal route of infection used in this study closely mimicked the natural intranasal route.

In this transmission experiment, the clinical syndrome displayed four phases distinguishable by practitioners in the field (13): an initial phase of normalcy in which the animal looked alert with a lustrous coat and no signs of disease; a subdued phase in which the animal looked less alert and showed signs such as a rough haircoat, increased rectal temperature, nasal and ocular discharges, dyspnea, submandibular swelling and reduced appetite; an apathetic third phase in which the animal responded rather slowly with coat losing luster in addition to symptoms of dyspnea, anorexia, extension of edema to dewlap and forelimb regions; and a final phase in which the animal showed a decrease in rectal temperature presumably owing to a loss of homeostatic control of temperature, severe edematous swelling of the submandibular-dewlap-forelimb, emaciation, tympany, recumbence and death. The course of the disease lasted between three and five days and there were various overlaps of the phases. The course of the disease generally recorded in natural outbreaks is however reportedly shorter than in experimental transmission suggesting that in natural field outbreaks the initial phases may escape notice (5).

The clinical and pathological findings in the challenged animals resembled the naturally occurring cases recorded by Bastionello and Jonker (2) for *P. multocida* type E:2 HS. A frequent clinico-pathology, which helps in the diagnosis of HS in cattle, is a subcutaneous edema with serosanguinous fluid in the throat-forelimbs

region, which also characterized the present findings. Gross pathological changes were largely limited to the thoracic cavity. Major differences were observed between the two calves: in one of them the lungs had generalized congestion with less complications; in the other the classical pneumonic form of HS was present, characterized by consolidation of the right apical lobe and adhesion of the right lung to the rib cage, depicting the diverse range of pathological features of HS seen in the field. De Alwis (5) showed that the extent of lesions depended on the duration of the disease. In peracute cases where death occurred in 24-36 hours, no more than a few scattered petechial hemorrhages were seen. When the course of the disease was more than 72 hours, there was extensive pneumonia, pleurisy and pericarditis with marked adhesions.

The challenged animals in this study developed septicemia as evidenced by the presence and re-isolation of the challenge organism from a variety of tissues and secretions. While it may be possible to isolate *P. multocida* from nasopharyngeal and saliva swabs of infected calves in the course of the disease, interpretation of the results should be tentative. It should be noted that *P. multocida* can be isolated as commensals from the intestinal and respiratory tracts of calves not suffering from pasteurellosis so the significance of the isolation of small numbers from these sites should be assessed in the light of pathological findings and the possible presence of other pathogens (8). A definitive bacteriological isolation can be made at autopsy from a range of numerous tissues and organs, namely, throat edema, lungs, lymph nodes, spleen, liver, and heart

blood of animals that succumbed to the acute form of the disease, as supported by the present findings. About 24 hours after death, *Pasteurella* could be isolated from the bone marrow tissue perhaps due to the rapid multiplication of the organism at tropical temperatures of 37-39°C.

■ CONCLUSION

From a diagnostician point of view, although a definitive diagnosis of HS is by bacterial isolation, a diagnosis based on clinical and morphologic criteria as described in this study might be helpful in cases where microbiological examination is either not possible because of submission of formalin fixed material, or because antibiotic treatment of the animal may have precluded isolation of microorganisms. The clinicopathological findings show that there are striking similarities between HS *P. multocida* types B:2 and E:2 (1, 5, 16). Appropriate comparative transmission studies are underway to compare the relative pathogenicity of the main three recognized etiological serotypes of *P. multocida* (B:2, E:2, and B:3,4) associated with HS in cattle.

Acknowledgments

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Résumé

Odugbo M.O., Turaki U.A., Itodo A.E., Okwori A.E.J., Yakubu R.A. Septicémie hémorragique (*Pasteurella multocida* sérotype E:2) expérimentale chez des veaux : étude clinique, pathologique et microbiologique

Une septicémie hémorragique (SH) a été induite expérimentalement chez des veaux de sept mois (n = 2) par inoculation intratrachéale de 10^{10} unités formant colonies d'une culture de *Pasteurella multocida* sérotype E:2 de 6 h en phase de croissance exponentielle pour étudier l'aspect clinicopathologique de l'infection et faire l'analyse microbiologique. La période d'incubation a duré quatre heures après l'infection. La série de signes cliniques généraux, classés par ordre de leur manifestation, ont été les suivants : fièvre, anorexie, dyspnée, gonflement de la région gorge-membres avants, tympanisme, jetage, salivation abondante, léthargie, décubitus et mort. A la nécropsie, les lésions prédominantes observées ont été : congestion pulmonaire avec symphyse pleurale des lobes craniaux, pleurésie, gonflement œdémateux du pharynx et du fanon avec exsudation d'un liquide jaunâtre semblable au sérum, et hémorragies dermiques et ecchymotiques. L'analyse histologique a révélé que les lésions pulmonaires étaient typiques de celles de la broncho-pneumonie fibrineuse avec épaississement des parois alvéolaires, congestion, œdèmes et réponse cellulaire des poumons. A la nécropsie, *P. multocida* sérotype E:2 a été à nouveau isolé bactériologiquement des poumons, des nœuds lymphatiques, du foie, des reins, de la rate, du fluide œdémateux et du sang cardiaque. Cet agent pathogène n'a pas été détecté dans le sang veineux jusqu'à quelques heures avant la mort. Les aspects cliniques et pathologiques observés sur les animaux ont montré des ressemblances frappantes avec *P. multocida* sérotype B:2 HS. Ces données devraient permettre aux vétérinaires de reconnaître les cas suspects de SH sur le terrain.

Mots-clés : Bovin – Veau – Septicémie hémorragique – *Pasteurella multocida* – Infection expérimentale – Nigeria.

Resumen

Odugbo M.O., Turaki U.A., Itodo A.E., Okwori A.E.J., Yakubu R.A. Septicemia hemorrágica experimental en terneros con *Pasteurella multocida* serotipo E:2: estudios clínicos, patológicos y microbiológicos

Se indujo experimentalmente una septicemia hemorrágica (HS) en terneros de 7 meses de edad (n = 2), por inoculación endotraqueal de 10^{10} unidades precursoras de colonias de *Pasteurella multocida* serotipo E:2 en fase log de 6 horas, con el fin de estudiar las características clinico-patológicas y microbiológicas de la infección. El período de incubación fue de 4 horas post infección. La continuidad general de los signos clínicos por orden de manifestación fue pirexia, anorexia, dispnea, inflamación de la región entre el miembro anterior y la garganta, timpanismo, descarga nasal, salivación profusa, letargia, decúbito y muerte. Las lesiones prominentes observadas durante la necropsia fueron congestión de los pulmones con consolidación y aderencias pleurales de los lóbulos apicales, pleuresía, inflamación edematosa de la garganta y epiglotis, con exudados amarillentos líquidos similares al suero, petequias y equimosis hemorrágicas. Histologicamente, las lesiones de los pulmones fueron típicas de una bronconeumonía fibrosa con septos alveolares gruesos, hipermia, edema y respuestas celulares de los pulmones. Durante la necropsia, *P. multocida* E:2 fue aislada de nuevo bacteriologicamente de los pulmones, linfonodos, hígado, riñones, bazo, fluido edematoso y sangre del corazón. El organismo no se detectó en sangre venosa hasta varias horas después de la muerte. Las características clínicas y patológicas observadas en los animales muestran grandes similitudes con *P. multocida* tipo B:2 HS. Estos datos deberían ayudar a los veterinarios a reconocer los casos sospechosos de HS en el campo.

Palabras clave: Ganado bovino – Ternero – Septicemia hemorrhágica – *Pasteurella multocida* – Infección experimental – Nigeria.

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