Bacillus anthracis as a cause of bovine abortion – a necropsy case requiring special biosafety measures

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Summary

In spring 2017, the first case of bovine anthrax in 20 years in Switzerland occurred in the canton of Jura. Carcasses of anthrax-deceased animals should not be opened due to the formation of highly resistant spores bearing the risk of environmental contamination and aerosolization. Nevertheless, in the course of this local outbreak, one sick cow from the affected farm, whose blood repeatedly tested negative for *Bacillus anthracis*, was necropsied after euthanasia under special biosafety precautions at the Institute of Animal Pathology, Vetsuisse-Faculty Bern. Necropsy revealed ventral edema, fetal death, necro-hemorrhagic placentitis and necrotizing iliac lymphadenitis. *Bacillus anthracis* was isolated only from placenta and altered lymph node. The biosafety measures taken during and after necropsy prevented a contamination of the necropsy environment, which was proven with bacteriological swabs. This case shows that anthrax may elicit unspecific symptoms mimicking other diseases, and veterinarians must be aware of these non-septicemic cases.

Keywords: Anthrax, *Bacillus anthracis*, biosafety, bovine abortion, disinfection, zoonosis

Bacillus anthracis als Abortursache beim Rind – ein Sektionsfall mit besonderen Anforderungen an die Biosicherheit

Im Frühling 2017 ist in der Schweiz erstmals nach 20 Jahren in einem jurassischen Milchviehbetrieb wieder Milzbrand aufgetreten. Da *Bacillus anthracis* nach Kontakt mit Luft hochresistente Sporen bildet, welche die Umwelt kontaminieren sowie Mensch und Tier infizieren können, sollten an Milzbrand verstorbene Tiere nicht eröffnet werden. Im Rahmen dieses lokalen Ausbruches wurde dennoch eine Kuh mit Fieber, aus deren Blut sich in wiederholten Kulturen keine Keime isolieren liessen, euthanasiert und am Institut für Tierpathologie der Vetsuisse-Fakultät unter speziellen Sicherheitsvorkehrungen seziert. Bei der Sektion konnte ein ausgedehntes subkutanes Bauchödem, eine abgestorbene Frucht im Uterus, eine hämorrhagisch-nekrotisierende Plazentitis und eine ebensolche sublumbale Lymphadenitis gefunden werden. Nach der Sektion durchgeführte bakteriologische Tupferproben bestätigten, dass mit den getroffenen Sicherheitsvorkehrungen eine Kontamination der Sektionsumgebung verhindert werden konnte. Dieser Fallbericht soll ins Bewusstsein rufen, dass auch Rinder an nicht-septikämischen Milzbrand erkranken können, welcher sich mit unspezifischen, harmloseren Krankheiten ähnelnden Symptomen und Läsionen manifestieren kann.

Schlüsselwörter: Milzbrand, *Bacillus anthracis*, Biosicherheit, Rinderabort, Desinfektion, Zoonose

https://doi.org/10.17236/sat00176

Received: 30.01.2018
Accepted: 07.06.2018

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Anamnesis

In April 2017, the Institute of Veterinary Bacteriology ZOBA, Vetsuisse Faculty Bern, isolated *Bacillus (B.) anthracis* from a bovine lung sample from a dairy farm in the canton of Jura, Switzerland. The private veterinarian previously collected this lung sample from a succumbed adult Montbéliard cow by necropsy on the premises. Following the Swiss Ordinance of Epizootic Diseases[9], the veterinary authorities placed a 2nd degree ban on the farm and prompted the temperature measurement twice daily from all remaining animals (30 dairy cows, 12 heifers, 20 calves). In the following 14 days, another four dairy cows on the farm developed fever ranging from 39.5 to 40.2 °C. However, repeated cultures of their blood performed at the ZOBA remained negative. While three bovines recovered without treatment, one 4.75-year-old Montbéliard cow showed persisting fever and signs of incipient abortion and was therefore treated once with penicillin G-procain (Procacillin®, MSD Animal Health GmbH, Weystrasse 20, 6006 Luzern, Switzerland, 0.1 ml/kg i.m.). In the meantime, the Jurassic veterinary authorities requested in consultation with the Bernese authorities a necropsy of this sick cow at the Institute of Animal Pathology (ITPA), Vetsuisse Faculty Bern. Sixteen hours after the antibiotic treatment, this cow was transported alive within a cattle carrier to the ITPA.

Biosafety measures taken before, during and after necropsy

For the necropsy, the ITPA's internal necropsy protocol for zoonoses was applied and extended with additional biosafety measures. The necropsy room was divided into three risk zones (Fig. 1A). The cow was delivered alive into the innermost zone, where tail vein blood was taken before euthanasia with pentobarbital (Esconarkon®, Streuli Pharma AG, Bahnhofstrasse 7, 8730 Uznach, Switzerland, 0.2 ml/kg i.v.). The blood was immediately sent to the ZOBA for preparation of smears and bacteriological cultures. Smears were stained with Giemsa's azure eosin methylene blue solution (Merck, Zug, Switzerland) and searched for the encapsulated rod-shaped bacteria typical for *B. anthracis*. Culture of whole blood was performed on tryptic soy agar plates containing 5% sheep blood (TSA-SB, Becton, Dickinson and Company, Allschwil, Switzerland) incubated at 37 °C for 18-24h.

After a negative blood smear evaluation, the necropsy was started in the innermost zone by two people equipped with disposable suits and arm protection sleeves (Tyvek® Classic Plus, DuPont™ de Nemours Sàrl, 2984 Luxembourg), triple gloves (nitrile and latex), HEPA-filtered respirators (Versaflo™, 3M™ Personal Safety Division, London ON, Canada), and sanitizable boots and aprons (Fig. 1B). A third person equally protected was responsible for sample packaging in the intermediate zone (Fig. 1A). As soon as available, samples from spleen, liver and kidney were submitted to the ZOBA for smear and culture preparation as described above. The necropsy was only continued after negative microscopic evaluation of the organ smears. Additional samples from placenta and iliac lymph nodes were harvested during necropsy and submitted to the ZOBA afterwards. Samples for histology were collected in 4% formalin and fixed for 72h.

Immediately after macroscopic evaluation and sampling of an organ system, tissues were soaked with the gluta-

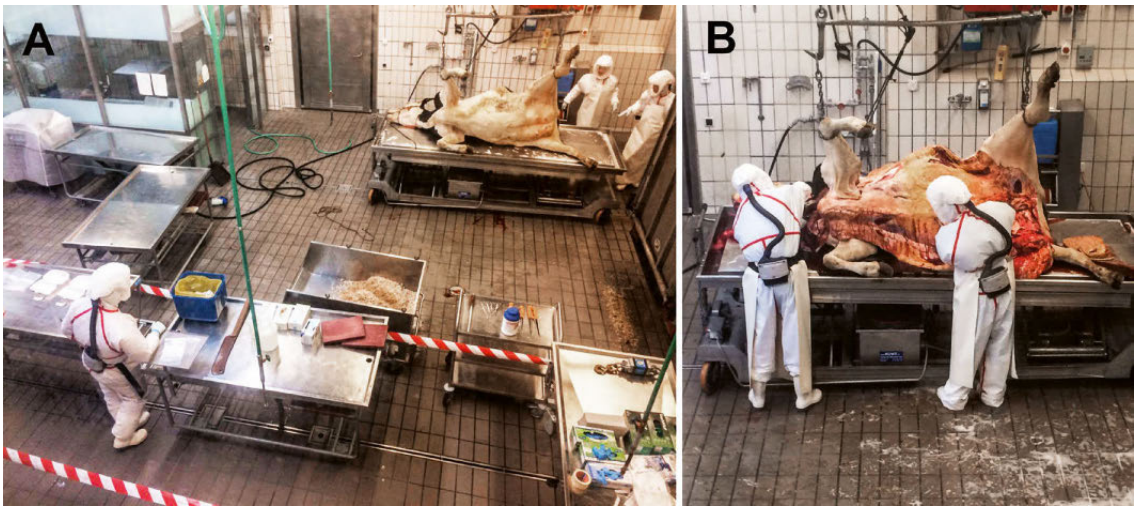

Figure 1: Necropsy room situation during the necropsy of the *B. anthracis* infected cow. The necropsy room was divided into three zones using movable necropsy tables and barrier tape (A). The cow was necropsied in left lateral recumbence by two persons (B), while a third person was responsible for sample packaging (A). All three persons wore disposable suits, triple gloves, arm protection sleeves, HEPA-filtered respirators, boots and aprons.

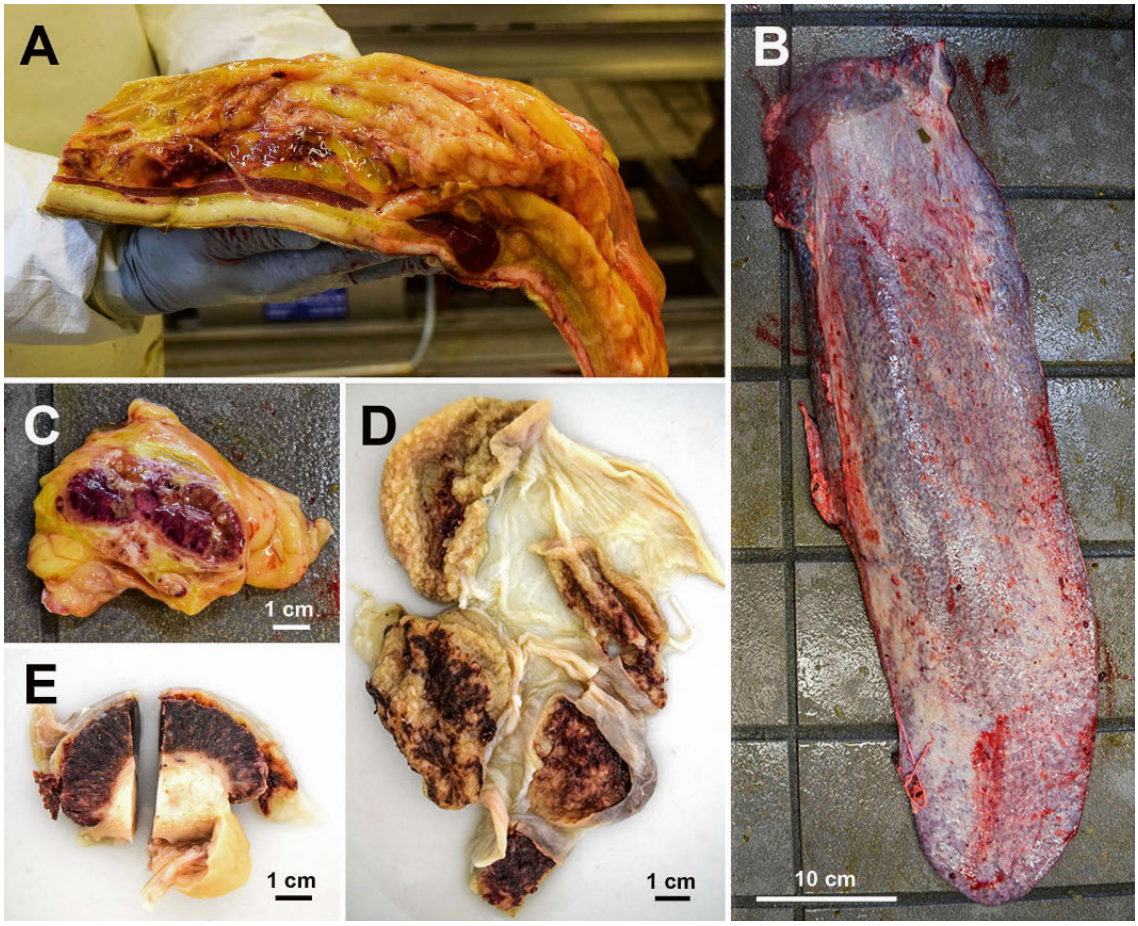

Figure 2: Macroscopic findings in the necropsy of the *B. anthracis*-infected cow. The cow showed a severe ventral subcutaneous edema and diffuse icterus (A). The spleen was without pathological findings (B). The iliac lymph nodes were severely enlarged and showed extensive hemorrhage and necrosis extending to the perinodal fatty tissue (C). Maternal and fetal placentome tissue (formalin-fixed) was hemorrhagic (D, E).

raldehyde containing reagent Neoform MED FF® (Dr. Weigert, Baarerstrasse 14, 6300 Zug, Switzerland) in a 4% dilution for 20 minutes before being transferred into the disposal container for potentially infectious animal by-products (Swiss category K1). Necropsy instruments and non-disposable protective clothing were immersed in 4% Neoform solution. The necropsy room was disinfected with 4% Neoform followed by cleaning with a chlorinated detergent (175-F,chl., Podchem, Härdlistrasse 2, 8447 Dachsen, Switzerland) twice within 2 h. The transport vehicle was disinfected using 4% Neoform, followed by an impregnation with 4% formalin (Formafix Switzerland AG, Stationsstrasse 3, 8335 Hittnau, Switzerland). The disposable protective clothing was autoclaved and incinerated.

The following day, the necropsy environment was repeatedly cleaned and disinfected. For final disinfection, 0.3% peracetic acid (Divosan activ®, Diversey Inc., 8310 16th Street, Sturtevant WI, USA) was used complying with the validated protocol for bacterial (*Geobacillus stearothermophilus*) spore inactivation under laboratory conditions from the Spiez Laboratory (Benjamin Weber, personal communication). After the disinfection procedure, a total of 47 bacteriological swabs were taken from the necropsy environment for analysis at the ZOBA by culture. Suspicious colonies were further analyzed by gamma phage lysis assay[2] and PCR specific for *B. anthracis*[8].

Pathological and bacteriological findings

The cow showed a mild reddish vaginal discharge and a diffuse swelling of the ventral abdomen consistent with a subcutaneous edema (Fig. 2A). The spleen was without findings (Fig. 2B). The iliac lymph nodes were severely enlarged and hemorrhagic (Fig. 2C). The dissection of the uterus revealed a dead edematous fetus and blood-tinged amniotic fluid (not shown). The placentomes were hemorrhagic (Fig. 2D and 2E) and the placenta detached easily.

Histological evaluation of the placentome tissue revealed extensive necrosis and hemorrhage (Fig. 3A). Numerous long bacilli were evident in the necrotic tissue in the HE and modified Gram stain (Fig. 3A and 3B). The iliac lymph node showed severe necrotizing inflammation (Fig. 3C), associated with hemorrhage, edema and vasculitis, but only few bacteria visible (not shown). Except for a mild embolic suppurative hepatitis (not shown), the other organs showed no histological lesions.

The bacteriological cultures from blood, spleen, liver and kidney remained sterile. However, an inhibitor test from the kidney sample performed in parallel according to the EU council directive 96/23/EG[3] and the EU regulation Nr. 854/2004[7] indicated the presence of antibiotics. Placenta and iliac lymph node contained large and small numbers, respectively, of bacteria suspected to be *B. anthracis*, demonstrated by Giemsa-stained smears and culture. *B. anthracis* isolation was subsequently confirmed by gamma phage lysis assay[2] (Fig. 3D) and by PCR specific for *B. anthracis*[8].

The culture and PCR analyses of the swabs taken from the necropsy environment after disinfection did not reveal any presence of *B. anthracis*. All persons involved in the necropsy and disinfection procedure thereafter remained healthy.

Literature overview and discussion

Anthrax is a worldwide occurring, usually fatal zoonosis of domestic and wild animals caused by *Bacillus anthracis*. This bacterium has the particular feature to form spores which are highly resistant to temperature, exsiccation and many disinfecting reagents, allowing survival in the environment for decades.[11] Herbivores and susceptible carnivores usually become infected by oral ingestion, rarely by inhalation, respectively, of spores from contaminated food and water.[10,11] The suspected minimal inhalational infectious dose in ruminants and humans ranges between 8'000 and 50'000 spores.[11]

In Europe, the disease is rare; however, it reappears sporadically in areas where anthrax-succumbed animals

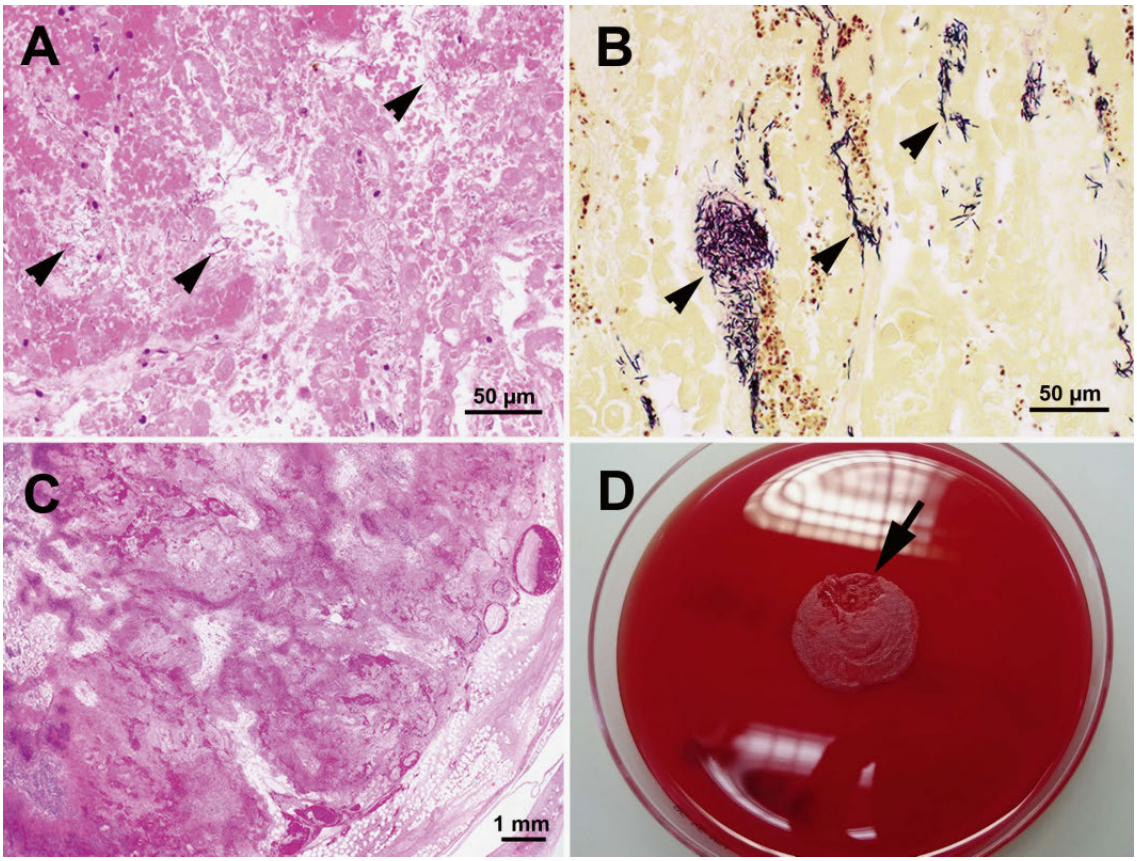

Figure 3: Histological and bacteriological findings from the *B. anthracis*-infected cow. The placentome tissue showed extensive necrosis and hemorrhage with faintly visible long bacilli present (arrowheads). HE, 400x (A). The modified Gram stain revealed numerous intra- and extravascular basophilic (= Gram-positive) bacilli (arrowheads) in the necrotic placentome tissue. Modified Gram stain, 400x (B). The iliac lymph node showed diffuse necrosis, hemorrhage and edema, affecting also the perinodal fatty tissue (lower right area). HE, 10x (C). *B. anthracis* culture with a lytic zone in the bacterial lawn (arrow) after gamma phage application (gamma phage lysis assay) (D).

have been buried or opened, or at former tanning or lane-processing industry sites.[1,11] The last bovine anthrax cases in Switzerland occurred in 1997 (canton of Schwyz) and 1993 (canton of Jura).[5] However, the 1993 Jurassian case occurred in another district than the case reported here.

Infected ruminants usually develop acute fever and massive bacteremia only a few hours before death.[6,10,11] Post-mortem findings are rapid autolysis, bloody discharge from body openings and a severely enlarged, dark spleen.[10] However, bovines may also suffer from a less acute disease with localized, bacterial exotoxin-induced necrosis and edema in the respiratory or gastrointestinal tract, as known from other species.[10,11] Abortions may occur; however, *B. anthracis* isolation from bovine abortion has been reported only once.[4,10] In the present case, an oral infection and hematogenous spread to the uterus is most likely. It can be hypothesized that the special physiologic conditions of the placenta favored a local bacterial proliferation, while a massive systemic bacteremia was prevented by the immune system.

The Swiss Ordinance of Epizootic Diseases Article 134 demands the euthanasia and the immediate disposal of anthrax-diseased animals without bleeding or opening the carcass to avoid the release of massive numbers of intravascular bacteria.[9] Article 132 defines an anthrax-diseased animal by isolation of *B. anthracis* from the blood. In the present case, however, *B. anthracis* was never isolated from the blood despite repeated blood cultures, justifying the request and the agreement to perform the necropsy. The farm's history requested special biosafety measures comprising protection of the necropsy personnel and prevention of environmental contamination. The latter was achieved by installing different risk zones in the necropsy room, by exclusion of a massive bacteremia in blood and tissue smears before and during necropsy, and, probably, by soaking the tissues with the glutaraldehyde containing Neoform MED FF®. Although this reagent is not approved for sporicidal disinfection, the application of the mycobactericidal 4% concentration immediately after organ evaluation most likely killed the vegetative bacteria and prevented spore formation.

It can be assumed that *B. anthracis* would have been present in the vaginal discharge and its immediate microscopic evaluation would have allowed an early tentative diagnosis and necropsy had been omitted. The Giemsa stain specifically stains the capsule of *B. anthracis*. Nevertheless, the evaluation of material by staining is less sensitive than culture. Therefore, the gold standard for diagnosis is culture supplemented with the gamma phage lysis assay or with PCR.[11] However, these methods require more than 12 h and are therefore not applicable for a timely exclusion of anthrax before and during a necropsy. Moreover, antibiotic pretreatment as in the present case may lead to false negative results.

Conclusion

This case shows that anthrax is still present in Switzerland. Practicing veterinarians, pathologists and laboratory personnel must be aware that affected bovines dot not always succumb to peracute septicemia. Anthrax must be considered a differential diagnosis when necro-hemorrhagic and edematous organ lesions are found. Not only blood, but all kinds of hemorrhagic discharge from body openings should be tested for the presence of *B. anthracis*. When a fast, highly sensitive and specific test to exclude anthrax immediately before or during a necropsy is lacking, necropsy of suspicious cases should be omitted for safety reasons or at least postponed until results from cultures are received. Development of a fast test, e.g. based on PCR or direct sequencing of clinical and organ samples is highly desirable. In this exceptional case, the extensive biosafety measures taken for the necropsy were sufficient to prevent personnel and environmental contamination despite unknown anthrax status during necropsy. Nevertheless, performing necropsies in case of anthrax suspicion should be discouraged.

Acknowledgements

We thank Gian Beer from the Nutztierklinik Bern for blood sampling and euthanasia of the sick cow. We thank Kathrin Summermatter from the Institute of Virology and Immunology for her advice regarding disinfection measures after the necropsy. We also thank Cornelia Staehelin from the University Clinic for Infectious Diseases, Inselspital Bern, for her guidance in the establishment of a medical emergency procedure. And we are much obliged to Paola Pilo from the Institute of Veterinary Bacteriology Bern who performed all the swab analyses after disinfection.

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Bacillus anthracis comme cause d'avortement bovin – un cas de nécropsie nécessitant des mesures particulières de biosécurité

Au printemps 2017, le canton de Jura a enregistré le premier cas d'anthrax bovin en Suisse depuis 20 ans. Les carcasses d'animaux morts d'anthrax ne doivent pas être ouvertes en raison de la formation de spores hautement résistantes présentant un risque de contamination de l'environnement et d'aérosolisation. Néanmoins, au cours de cette épidémie locale, une vache malade de l'exploitation touchée, dont le sang avait, de façon répétée, été testé négatif à *Bacillus anthracis*, a été autopsiée après euthanasie avec des précautions spéciales de biosécurité à l'Institut de pathologie animale de la faculté Vetsuisse de Berne. La nécropsie a révélé un œdème ventral, un foetus mort, une placentite nécro-hémorragique et une lymphadénite iliaque nécrosante. *Bacillus anthracis* a été isolée uniquement à partir du placenta et des ganglions lymphatiques modifiés. Les mesures de biosécurité prises pendant et après la nécropsie ont empêché la contamination de l'environnement, ce qui a été prouvé avec des prélèvements bactériologiques. Ce cas montre que l'anthrax peut provoquer des symptômes non spécifiques imitant d'autres maladies et que les vétérinaires doivent être conscients de ces cas non septicémiques.

Mots clés: Anthrax, *Bacillus anthracis*, biosécurité, avortement bovin, désinfection, zoonose

Aborto bovino causato da *Bacillus anthracis* – un'autopsia che richiede speciali misure di biosicurezza

In Svizzera, nella primavera del 2017 si è verificato nel cantone Giura il primo caso di carbonchio bovino in 20 anni. Le carcasse di animali deceduti a causa del carbonchio bovino non devono essere aperte a causa della formazione di spore altamente resistenti che comportano rischi di contaminazione ambientale e di aerosolizzazione. Tuttavia, nel corso di questa epidemia locale, un animale malato proveniente dall'azienda colpita e il cui sangue è risultato ripetutamente negativo al *Bacillus anthracis*, è stato sottoposto, dopo l'eutanasia, a autopsia con speciali precauzioni di biosicurezza presso l'Istituto di patologia animale della Facoltà Vetsuisse di Berna. L'autopsia ha rivelato un edema ventrale, una morte fetale, una placentite necro-emorragica e una linfadenite iliaca necrotizzante. Il *Bacillus anthracis* è stato isolato solo nella placenta e nei linfonodi alterati. Le misure di biosicurezza adottate durante e dopo l'autopsia hanno impedito una contaminazione dell'ambiente, che è stata comprovata con tamponi batteriologici. Questo caso dimostra che l'antrace può provocare sintomi non specifici che imitano altre malattie e i veterinari devono essere consapevoli di questi casi non setticemici.

Parole chiave: Antrace, *Bacillus anthracis*, biosicurezza, aborto bovino, disinfezione, zoonosi

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