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Review Article

A Short Review on Blackleg in Ruminants

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Abstract

Blackleg is an endogenous acute infection that principally affects ruminants. It is *Clostridium chauvoei*, anaerobic spore-forming bacteria, causes the disease, which manifests as an acute, localized inflammation of muscle tissue produced by development of the blackleg organisms. Although blackleg may occur at any time of year, more cattle are lost during the summer months. In recent years, the major virulence factors of *C. chauvoei* have been discovered and described. However, the pathogenesis of blackleg in ruminant, and in particular the pathogen's movement from the place of entrance to the affected tissues is yet unknown. When outbreaks arise, it is prevented by vaccination; in the early stages of the disease, it is treated with antibiotics, most notably penicillin, which is an effective therapy for the condition. Control of this disease is based on strict husbandry methods and a vaccination program. The key virulence factors of *C. chauvoei* have been found and characterized in recent years. This review covers the most recent research discoveries that contribute to a better understanding of the disease and provides the basis for preventative efforts.

Keywords: *Clostridium chauvoei*; Myonecrosis

Introduction

Blackleg (Black quarter, Quarter evil, Rausch brand, Charbon symptomatique) is an economically important disease with high mortality that affects cattle, sheep and other animals [1]. *Clostridium chauvoei*, the etiologic agent of blackleg, is a gram-positive, motile, histotoxic, and sporulating anaerobic bacterium. Ingestion of *C. chauvoei* spores is probably the most common form of exposure and infected ruminants do not directly transmit the disease to other animals [2].

The end spores of *C. chauvoei* can lie dormant in the soil for years and, after ingestion by the animal, they are assumed to cross over the gastro-intestinal tract, enter the bloodstream and finally migrate in various organs and muscles, where they remain dormant until stimulated to cause disease [3]. The typical infection in ruminants is characterized by my necrosis of striated and cardiac muscle and often per acute death. A unique feature of the disease in cattle is the appearance as a non-traumatic endogenous infection [1].

Clostridium chauvoei has a tiny genome compared to other Clostridium species, such as *C. difficile*, with just 4.2 million base pairs [4]. This indicates its adaption to a limited host range (bovine, caprine, and ovine) where it can replicate and cause disease [5]. Even though blackleg is one of the oldest known diseases affecting ruminants, there are important gaps in the understanding of this disease, especially with respect to its pathogenesis. Focusing on the ruminant disease, this article aims to offer an overview of the current knowledge about the etiology, virulence factors, epidemiology, pathogenesis, diagnosis, and provides a foundation to preventive strategies.

3. Epidemiology Clinical and Pathological Manifestation

Although blackleg is usually a disease of pastured cattle, it may also affect sheep. It mostly affects animals under the age of two, with the majority of cases occurring in cattle from 4 to 24 months [6]. Occurrence of the disease is worldwide, but, it tends to be localized, even to certain farms or to certain pastures. Because of this localization, it is assumed that *C. chauvoei* is soil borne, but likely does not grow in soil. Once exposed to the environment, *C. chauvoei* readily forms spores, which may survive for long periods (many years) in the soil [7]. Despite the fact that the disease is a soil-borne infection, the organisms enter the body through the alimentary mucosa after consuming contaminated feed. True black leg develops when spores which are lodged in normal tissue and proliferate by mechanisms such as trauma or toxemia [8]. The bacteria grow readily in the intestinal tract of cattle and may be recycled through fecal contamination of the soil. The incubation period of blackleg may either be 1–5 days or 2–5 days [9,10]. The clinical signs in the per acute form of the disease are so short-lived that they are usually not observed [11].

The acute form of the disease is the most commonly reported.[12] The observable clinical signs associated with blackleg in cattle include marked lameness with pronounced swelling of the affected limbs, marked depression, anorexia, ruminal stasis, high pulse rate (100–120/min), acute rumen tympany, high temperature (41^{0}C) [13] protruding tongue, tongue and throat swelling; edema, emphysema and crepitation of affected heavy muscles, marked respiratory distress, stiffness of thigh muscles and reluctance to move; and discolored, dry and cracked skin [14].

The clinical signs of the disease in sheep are the same as in cattle, except crepitation may not be palpable in the swelling during life [13]. Lesions resulting from chauvoei infection are typically within the larger muscle groups of the limbs. The affected area is dark red, within which small areas of necrosis may be observed [15]. True blackleg is most commonly linked with cattle and sheep, however outbreaks have been observed in deer and, in one occasion, a horse. The disease typically affects young cattle aged 6 months to 2 years. The disease appears to be more prevalent in cattle that are rapidly growing and receiving a high level of nutrients in the field. Increased protein

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feeding improves sheep nutrition, making them more vulnerable to blackleg. When it comes to sheep, there are no age restrictions [16].

The typical blackleg of cattle has a seasonal occurrence, with the majority of instances occurring during the warm months of the year. The peak frequency may fluctuate from spring to fall, most likely dependent on when calves enter the vulnerable age range. Some outbreaks of blackleg in cattle have occurred following soil excavation, suggesting that soil disturbance may reveal and activate latent spores [17].

Etiology and Virulence Factors

Blackleg is caused by *C. chauvoei*. The organism was named after Professor J. A. B. Chauveau, a French bacteriologist [18]. Clostridium chauvoei the causative agent for blackleg is an anaerobic, highly pathogenic, endospore forming and gram-positive bacterium, which produces lemon-shaped endospores and requires enriched media for growth [2]. The spores are highly resistant to environmental changes and disinfectant and persist in soil for many years and the organisms are typically pleomorphic. False blackleg may be caused by Cl. Septicum and Cl.

Novyi but this disease is more accurately classified as malignant edema. In 2013, the first draft genome sequence of a virulent *C. chauvoei* strain became known, consisting of 2.8 million base-pairs [19]. Moreover, it contains a cryptic plasmid, about 5.5 kbps in size [5].

The entire genome sequences of 20 strains of *C. chauvoei* collected over a 64-year span from four continents were found and examined. The findings of this study indicated that the strains investigated were highly conserved, indicating that *C. chauvoei*'s evolution had come to a standstill [20].*C. chauvoei*'s genome is small in comparison to other Clostridium species, such as *C. difficile* (4.2 million bp) [4], suggesting the organism's adaptation to a narrow host range (bovine, caprine, and ovine), where it may grow and cause disease [5]. *C. chauvoei* genomes isolated in Germany revealed unique mutations in regulatory genes, showing that *C. chauvoei* has particular control over regulatory events, in contrast to the genomes of other Clostridium species [1].

Clostridium chauvoei has 69 genes dedicated to the mechanisms involved in sporulation and dormancy [5], which might be thought of as virulence characteristics that allow the disease to withstand harsh environmental circumstances and stay potentially infectious for years. It was recently discovered that the genes involved in sporulation and germination in *C. chauvoei* are identical to those found in Clostridia cluster I, which contains *C. botulinum*, *C. haemolyticum*, *C. novyi*, *C. perfringens*, *C. tetani*, *C. septicum*, and *C. chauvoei*. Furthermore, *C. chauvoei* generates a number of cellular associated virulence (somatic and flagellar) and soluble antigens [2].

Cellular Antigens

The bacterial cell incorporates Somatic antigens. Such antigens are thought about crucial immunogenic components involved in the protection against *C. chauvoei* infection, and so, current and previous vaccines contained bacterins or were alone composed of bacterins [21]. Flagellar antigens are studied extensively; highlight flagellin that is encoded by the fliC sequence. Flagellin features a pathogen-associated molecular pattern (PAMP) that's recognized by toll like receptor five (TLR5) expressed by monocytes and fibroblasts. The receptors at the surface of viscous animal tissue cells bind the preserved regions of flagellin (N and C terminals), leading to the activation of protein secretion [22].

More studies characterized flagellin and evaluated its protecting activity by employing a recombinant flagellin super molecule. These authors' reportable poor protecting immunity evoked by the recombinant flagellin in mice, suggesting that a conformation-dependent epitope plays a very important role within the development of immunity against blackleg. The poor protecting activity of the recombinant flagellin super molecule determined antecedently [23] are often attributed to the actual fact that these authors failed to thought-about that their square measure a minimum of 2 copies of fliC sequence on the body of *C. chauvoei* [24]. [20] Found 3 copies of the cistron variants fliC1, fliC2, and fliC3 of flagellin in most strains studied, so showing ninety-one.8% organic compound identity with one another in an exceedingly given strain and 82-96% identity between the paralogues of various strains. [1] Conjointly discovered the presence of 3 fliC genes. The cell surface-associated antigens of *C. chauvoei*, aside from flagellin, haven't nonetheless been explored. [25] Known some necessary cell surface associated proteins of *C. chauvoei*, like enolase, chaperonin, ribosomal supermolecule L10, flavoprotein, and glycosyl hydrolase, that showed protecting antigenicity in different bacteria. However, further studies are necessary to judge the role of those surface-associated proteins in protection against blackleg.

Soluble Antigens and Toxins

Toxins (soluble antigens), are deeply involved in the pathogenesis of blackleg. The hemolytic leukocidin CctA, oxygen-labile hemolysin D (or hemolysin III), DNase (ß-toxin), hyaluronidase Nag (previously called γ-toxin), and neuraminidase/sialidase NanA are the five *C. chauvoei* toxins are known at present. A thermostable protein responsible for the nuclear degradation of muscle cells DNase (ß-toxin) is an enzyme of the deoxyribonuclease type and participates in clostridial myonecrosis [26].

It was found in >80% *C. chauvoei* strains isolated from cattle, although the strains showed different capacities of toxin production. *C. chauvoei*'s full genome study revealed the existence of two genes encoding the large and small subunits of exo-deoxyribonuclease VII, which most likely reflect *C. chauvoei*'s DNase activity [19]. The genes producing exo-deoxyribonuclease VII are present and completely conserved in all 20 *C. chauvoei* strains [20]. Hyaluronidase (γ-toxin) is a heat-inactivated enzyme that can degrade hyaluronic acid. It is thought to be responsible for destroying the loose connective tissue that surrounds the muscles, allowing *C. chauvoei* to spread throughout the tissues of the infected host [26].

The spore-forming, oxygen-stable leukocidin hemolysin called *C. chauvoei* cytotoxin A (CctA) confers strong hemolytic activity, which is observed as a halo around the colonies on blood agar growth medium. CctA as a mature protein has a molecular mass of 32.2kDa. It is a major toxin and hemolysin produced by *C. chauvoei*, which is shown to be highly cytotoxic to the bovine epithelial cell line ECaNEp [27]. In addition [27], used the conventional assay for testing the potency of blackleg vaccine, which contains purified recombinant

CctA as the sole antigen, and protects 80% guinea pigs from the challenge with virulent *C. chauvoei*. The antibodies directed against CctA play the main role in the protective immunity exerted against blackleg; thus, it is a valuable candidate for blackleg vaccines and for the potency testing of current vaccines.

The previously described oxygen-stable necrotizing hemolysin (a-toxin) might be CctA, although the reported molecular mass of this a-toxin hemolysin is 25kDa, which is significantly lower [28]. Alternatively, this a-toxin could represent the putative hemolysin III, also called hemolysin D or d-toxin (protein #276) found on the genome of *C. chauvoei* [5] whose has around 25kDa molecular mass. It should be emphasized that hemolysin III is not unique to *C. chauvoei*, since it is expressed or carried by various pathogenic, commensal, and environmental gram-positive bacteria. Although there is no clarity about hemolysin III in *C. chauvoei*, it is reported to be similar to the θ-toxin produced by *C. perfringens* and the tetanolysin produced by *C. tetani* [26,27].Used monospecific antibodies directed against CctA to completely neutralize all of the hemolytic activity expressed by *C. chauvoei*, demonstrating that this pathogen does not create any entity with quantifiable hemolytic activity other than CctA.

Characterized Neuraminidase/sialidase (NanA) in detail as an 81-kDa protein that is secreted as a dimer [27]. The nanA gene, which has been entirely conserved across *C. chauvoei* strains isolated over 60 years from various geographical locations across four continents, encodes it [20]. A recombinant molecule derived from *nana* containing the sialic acid-binding domain (CBM40) is able to fully neutralize the sialidase activity of *C. chauvoei* [6]. Thus, NanA can also be used as a potential antigen to aid protective immunity

Pathogenesis

There is no consensus on the pathogenesis of blackleg [29]. The spores are swallowed from the soil, enter the gastrointestinal tract, and travel to the muscle through the hematogenous pathway, where they remain latent in cells of the mononuclear phagocytic system. The spores might be latent in the muscle for years [25]. Transient trauma or ischemia promotes spore germination and the production of cytolytic toxins that cause necrosis of vascular endothelia (edema, hemorrhage) and myofibers. Toxins are taken into the animal's circulation, where they induce acute sickness and death. Clostridial proliferation produces gas that appears as bubbles between muscle bundles [30].

The vegetative forms of the organism enter the circulation before localizing in injured or poorly drained muscles [9]. Another report suggests that the infection is acquired by ingestion of spores, which are probably taken across the alimentary mucosa in macrophages and then distributed to the tissues including muscles [13]. The spores live in the muscle as a dormant infection and become active and vegetative when the muscle is wounded in some way, reducing its oxidation–reduction potential. This leads to anaerobiosis and lowered hydrogen ion concentration (pH) in the muscles, thereby creating a good environment for the germination of the spores to produce blackleg. High rainfall is a known predisposing factor to blackleg as the spores are dispersed to far areas to become vegetative and infective during grazing [27]. Soil excavation has been reported to also predispose ruminants to blackleg [31]. Vaccination could also be a source of trauma which may predispose to infection, by creating a favourable condition for the growth of latent spores through needle punctures in muscles [32].

Prevention and Control

If antibiotics are not given, new cases of black leg may arise for up to 14 days as unit immunity develops, necessitating continual surveillance and early treatment of cases [33]. On farms where the disease is prevalent, all cattle between the ages of 6 months and two years should be vaccinated yearly, right before the expected risk season, which is generally spring and summer. When the disease is prevalent, calves should be vaccinated at 3 weeks of age, and animals should be moved away from the infected area. Bacteria prepared from C1. Chauvoie is preferred. The expected improvement would be even larger if the toxin composition of each isolate was known rather identifying only its identifying antigenicity. Cattle between the ages of 3 and 6 months have to be vaccinated, and then every year after that [32].

Vaccinations for cattle are also made with attenuated organisms, and certain attenuated bovine strains or freshly identified, virulent bovine strains may be utilized to make vaccines for sheep. 32 The use of a polyvalent vaccination is strongly recommended for the additional protection it provides at a low cost. Wind, rain, and scavengers can disperse large amounts of spores. In locations where the disease is known to exist, blackleg vaccination should be a common approach on all properties. The vaccination should be administered under the skin on the side of the neck to prevent infection from spreading to the muscles [33].

Susceptible cattle, who must be vaccinated in endemic regions, should also be immunized on a regular basis because of the possibility of infection being introduced with car cases of dead cattle, which may be dumped during flooding. When beef calves are branded and have their ears marked, they are normally vaccinated around one or four months of age [32]. A booster immunization given a month or two after weaning is usually enough to protect until age immunity takes over at around 24 months of age. Calves that were vaccinated before the age of three months should be revaccinated between the ages of four and six months, and again at weaning [34].

Conclusion

Blackleg is an acute and often fatal infection occurring in cattle that continues to remain endemic worldwide despite large vaccination programs. Studies characterizing cellular and soluble antigens are necessary to improve the chances of developing a protective vaccine. I also want to highlight that the commercial vaccine are bacterins that are probably ineffective in extending immunity against *C. chauvoei* spores, sialidase, and CctA.

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