Bovine Antibody Response to Respiratory Disease Caused by Mannheimia haemolytica Leukotoxin

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Abstract

In order to find out Mannheimia haemolytica was involved in outbreaks of bovine respiratory disease (BRD), a serological survey was done on paired (acute-convalescent) sera from 200 beef cattle and 250 dairy cattle during 10 outbreaks of BRD in Pakistani herds between 2021 and 2022.The sera were collected during BRD outbreaks in Pakistan. In the herds that were examined, there was no evidence of a vaccination programme against M. haemolytica A1. For each epidemic, a serum sample from five to ten animals was evaluated for collection. In order to evaluate the antibody response of the serum to M. haemolytica leukotoxin, an enzyme-linked immunosorbent test, or ELISA, was carried out (LKT). The seroconversion process was carried out on animals (54%), specifically 70 beef cattle (28%) and 55 dairy cattle (27%), respectively. It was determined by serological testing that M. haemolytica was involved in 10 (9%) of the BRD outbreaks. It ranged from 10% to 40% range for the prevalence of seroconversion. In 200 out of 510 total cases, there seemed to be evidence of a concurrent seroconversion to M. haemolytica and the primary bovine respiratory viruses. Cattle that had not been vaccinated against BRD viral agents were the most likely to develop antibodies against M. haemolytica LKT.

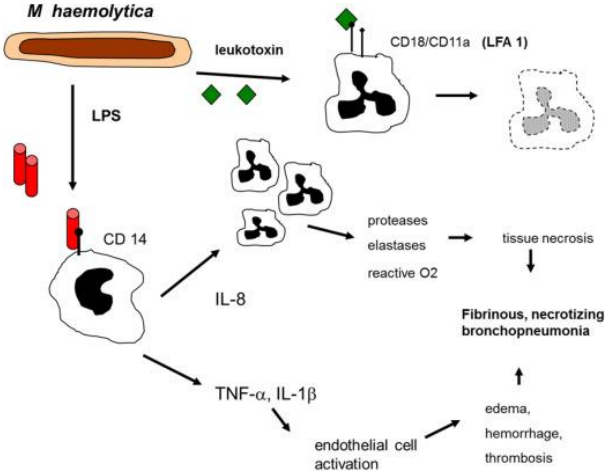
Keywords: *Bovine, Mannheimia haemolytica, leukotoxin, antibodies.*

INTRODUCTION

Bovine respiratory disease (BRD) is the most common and costly beef cattle disease worldwide. Complex bacterial or viral pneumonia in calves can be fatal. Stress, viral infection, and new bacterial infection usually cause the infection. Multiple causes complicate disease diagnosis. BRD, also known as "shipping fever," usually strikes calves four weeks after weaning when they are sorted and sold to different farms. It is not clear whether the most significant contributing factor is stress, co-mingling, or travel conditions (Frank et al., 1989). There is no evidence that can be considered conclusive regarding more specific factors; however, studies have identified general stressors such as transportation and cold weather (e.g. distance, transport mode, temperature, or temperature volatility). Pneumonic pasteurellosis (also known as shipping fever) is a significant component of the bovine respiratory disease (BRD) complex. Despite its name, Mannheimia haemolytica is not harmful to healthy cattle (Mosier et al., 1997). Fibrous pneumonia is an illness that may affect newborn calves (enzootic pneumonia). In young calves (enzootic pneumonia), animals that have been weaned from beef (shipping fever), and dairy cattle, it is thought to cause

fibrinous pneumonia. Most deaths from BRD are caused by M. haemolytica-related fibrinous pneumonia. In most cases of bovine pneumonia, M. haemolytica serotype A1 is the causative agent (Mosier et al., 1997). The microorganism M. haemolytica A1, which normally lives in the tonsillar crypt as part of the normal microbial flora of healthy cattle, rapidly replicates in response to the presence of stress factors, colonies the nasopharynx, and is transported to the lung via aerosolized droplets Leukotoxin is produced by the organism while it is in the logarithmic growth phase (LKT) (Whitely, et al., 1992). Recent research suggests that M. haemolytica LKT's activation and lytic activity may be a result of binding to a specific site on bovine leukocytes. Fibrin deposition may be amplified by M. haemolytica LKT because it causes bovine alveolar macrophages to surface-express fibrinolytic activity (Bowersock et al., 2014).Serum antibody responses can be triggered in cattle by LKT following natural exposure to the microorganism and also following treatment with vaccines containing LKT (Dassanayake, et al., 2013). Considering that the production of LKT is connected to the active replication of M. haemolytica, the detection of a specific antibody response is an option to take into consideration (R. P, et al., 2013). Presentation of its involvement in respiratory disease, albeit in an indirect manner. Both the labor-intensive cytotoxin neutralisation assay (LKT) and the enzyme-linked immunosorbent assay (ELISA) have been used to measure antibody responses (Orouji, et al., 2013). Previous isolation in cultures in Pakistan has provided evidence that M. haemolytica was involved in BRD outbreaks in that region. This evidence was obtained in Pakistan. Since it is challenging to get appropriate samples (nasal swabs or lung tissue) from acutely infected animals, the contribution of M.haemolytica to respiratory outbreaks in cattle may be underestimated (Batra, et al., 2016). Serological studies to detect seroconversion to M. haemolytica LKT could, therefore, help clarify the incidence of pneumonic pasteurellosis among BRD outbreaks (Taylor, et al., 2010). Serum pairs were collected from beef and dairy animals in areas experiencing BRD epidemics for a serological study. The research was conducted to learn more about M. haemolytica's role in the current BRD outbreaks in Pakistan.

Antibody Response to Mannheimia Haemolytica Leukotoxin



Materials and methods

Animals

Serum samples were taken from 200 beef cattle and 250 dairy cattle in 60 BRD outbreaks that happened in Pakistani herds between 2021 and 2022. Most of the time, nasal and eye discharge, fever, coughing, and breathing difficulties were used to make a clinical diagnosis of BRD. In the flocks that have been looked at, there was no M. haemolytica A1 vaccination programe (Li, et al., 1999).

Serum Samples

For each BRD outbreak, serum was taken from anywhere from 5 to 10 animals. Before being used, sera were frozen at –20°C. Which utilized in viral neutralisation and hemagglutination experiments were killed by heat at 56°C for 30 minutes.

Serology

To find out how serum antibodies react to M. haemolytica LKT, an indirect enzyme linked immunosorbent assay (ELISA) was used. The antigen for ELISA was a purified version of LKT that was made using method with a few small changes. In brief, M. haemolytica A1 reference strain ATCC BAA-407/DSM 14655 was cultured in l logarithmic-phase medium at 37°C in a shaking incubator, and the LKT was isolated with 40-60% ammonium sulphate from the supernatant. The precipitate was dialyzed against phosphate buffered saline in 10 ml of 3M guanidine, 50 mM NaHPO4, 100 mM NaCl (PBS).Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) with 4% stacking and 10% resolving gels was used to determine the presence of LKT (Herndon, et al., 2010). To determine the molecular weight of the obtained LKT, the gel was electrophoretically blotted onto an Immobilon-P (Millipore) membrane. After blotting, the membrane was probed with a monoclonal antibody (MAb) that was specific to M. haemolytica A1 LKT. The blocking step consisted of adding 3% gelatin to a solution of tris-buffered saline (TBS). Membrane was rinsed, then incubated with goat anti-mouse immunoglobulin G coated with horseradish-peroxidase (Sigma). The substrate diaminobenzidine was used to develop the immunoblot (Sigma). We determined the molecular weight of the LKT band to be around 100 kDa by using immunoblotting. Additionally, all matched serum samples were analysed for anti-Bovine Herpesvirus 1 (BHV-1), anti-Bovine Viral Diarrhea Virus (BVDV), anti-Bovine Coronavirus (BC), anti-Bovine Respiratory Syncytial Virus (BRSV), and anti-Parainfluenza 3 (PI3) antibodies using either a virus neuralization test (VN), an indirect fluorescent antibody assay (IFA), by hemagglutination inhibition test (HI). Seroconversion was proven in every case by the above said measures (Cai, et al., 2010).

RESULTS

Table 1 shows the results for M. haemolytica seropositivity to LKT in paired serum samples from cattle with respiratory disease. At the first test (acute serum), 10% of the 510 cattle that were tested had antibodies to LKT. Seroconversion involved 120 animals (9%) in 62 outbreaks (31%) became seropositive. Of these, 80 beef cattle (40%) in outbreaks 40 (20%) and 130 dairy cattle (52%) in outbreaks 80 (32%) became seropositive 20%–70% of BRD outbreak animals seroconverted to M. haemolytica. Beef cattle had a greater seroconversion rate (30% for the animals and 70% for the outbreaks) than dairy cattle (15% for the animals and 85% for the outbreaks) ($p<0.01$ and $p<0.05$, respectively). 150 (70%) calves and heifers and cows 63 (42%) seroconverted to LKT in dairy cattle (Li, et al., 1999). M. haemolytica LKT and viral seroconversion occurred simultaneously in 5.8% of animals and 5.0% of 120 outbreaks. Concurrent seroconversion to M. haemolytica LKT and other viruses was seen for BRSV (n=10), BVDV (n=14), BC (n=15), PI3 (n=20), and BHV-1 (n=20). Antibodies against M. haemolytica LKT and other respiratory viruses were frequently found in the same animal (n = 27).Outbreaks with simultaneous seroconversion and LKT seroconversion alone both had a mortality rate of 12%, while the

latter only had a mortality rate of 10%. In a study of 510 animals who had seroconverted to M. haemolytican LKT, 30%, or 155 animals, had not been vaccinated against viral respiratory infections such as BHV-1, BRSV, BVDV, or PI3. Bovine Herpesvirus 1 (BHV-1), Bovine Viral Diarrhea Virus (BVDV), and Bovine Coronavirus (BC) were examined using a virus neutralisation test (VN), Bovine Respiratory Syncytial Virus (BRSV) was analysed using an indirect fluorescent antibody assay (IFA), and Parainfluenza 3 (PI3) was examined using a hemagglutination inhibition test (HI). Seroconversion was proven in every case by the aforementioned measures.

Table 1. Antibody Response to M. haemolytica Leukotoxin (LKT) in Serum Samples from Cattle with Acute Respiratory Disease

Animal category	Examined	Seroconversion*
Beef cattle Animals Outbreaks	200 60	145 (72%) 55 (27%)
Dairy cattle Animals Outbreaks	250 37	180 (72%) 70 (28%)

DISCUSSION AND CONCLUSION

Our field study confirmed that M. haemolytica causes bovine respiratory tract illness, which was already known from bacterial isolation data in BRD outbreaks in cattle herds in the same location 10. (Midkiff, et al., 2021). The immunological response to LKT demonstrated that M. haemolytica was actively reproducing in most cattle after acute respiratory episodes for a time. M. haemolytica causes respiratory episodes by seroconverting to LKT in paired (acute-convalescent) serum samples M. haemolytica has a short colonization phase. Which makes it hard to get bacteria from nasal swabs, serological testing could help make a better diagnosis. The frequency of individual seroconversion to M. haemolytica LKT in our survey ranged from 20% to 60%, with 22% of the affected cattle belonging to 62% of the BRD outbreaks testing positive for seroconversion. The serological evidence indicated that M. haemolytica was actively infecting the BRD epidemics that were studied. Our findings show a high rate of concurrent seroconversion to M. haemolytica LKT and viruses, suggesting that the disease may be caused by M. haemolytica of the viruses associated with the investigated BRD outbreaks, BRSV was the one with the highest prevalence of seroconversion. Remarkably, the most often recognized causes of enteric disease BVDV and BC were also associated with a high proportion of seroconverted animals. On the other hand, both viruses have been linked to respiratory outbreaks in Pakistan. It is common knowledge that infection with BVDV can lead to a respiratory illness. Damage to the lung tissue is caused by the replication of BVDV in the mucosal cells that line the respiratory tract. In addition, immunosuppression that occurs after a BVDV infection makes it more likely that the respiratory tract will become colonised by other viruses and bacteria, including M. haemolytica. It was determined that the presence of coronavirus strains with tropism and pathogenic activity for the respiratory tract of cattle was responsible for the role that BC played in the induction of BRD. Our research shows that the pathogenicity of M. haemolytica is elevated when the bacterium is co-infected with viruses. This is supported by the fact that the majority of cattle that developed a seropositive response to LKT were not previously immunised against BRD viral

agents. As a result, vaccination against bovine respiratory viruses, including BVDV, also aid in the fight against the M. haemolytica infection. Therefore, it is important to consider the lack of a virus immunization programme to be a risk factor for the development of M. haemolytica fibrinous pneumonia. Antibody detection against LKT has been shown to be useful in assessing the presence of an active infection. The severity of fibrinous pneumonia can be prevented or considerably reduced by using LKT antibodies. LKT antibodies protect against live M. haemolytica intratracheal or transthoracic intrapulmonic challenge, according to virulence factor studies. The capacity of the antibody to neutralise the LKT protein is what provides the protection. This has led to M. haemolytica A1 LKT being included in commercially accessible vaccination formulations for the cattle industry. The significant prevalence of concurrent M. haemolytica and viral infections suggests that beef and dairy cattle should be routinely vaccinated against viral infections and pasteurellosis. As shown in study, these mixes usually yield positive results. The majority of seroconversions in dairy cattle were in calves, which must be considered when determining the best age for vaccination. Research suggests that dam vaccination, which boosts specific passive immunity, could minimize pneumonia in young animals. Our data on BRD after shipment and field trials across a large geographic range of locations, types, and sources of animals under different managements suggest that vaccinating beef cattle at the feed yard reduces respiratory disease mortality (Li, et al., 1999).

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