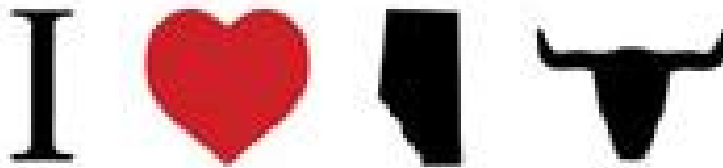


BRD in feedlots: comparison of laboratory versus cowboy diagnostic methods



Research Internships report Maarten van den Bosch
October – December 2010
Department of Production Animal Health
Faculty of Veterinary Medicine
University of Calgary, Alberta, Canada

Contents

| | |
|---|----|
| Front page | 1 |
| Contents | 2 |
| Introduction | 3 |
| Feedlot system | 3 |
| Research project | 3 |
| BRD | 4 |
| Research internship | 4 |
| Selection of laboratory parameters | |
| Disease caused by infectious agents (BRD) | 5 |
| Disease caused by non infectious reasons (ruminal acidosis) | 5 |
| Acute Phase Proteins | 6 |
| Major, moderate and minor APP's | 7 |
| Functions APP's | 7 |
| Bovine APP's | 7 |
| Laboratory Haptoglobin tests | 9 |
| Field trial | |
| Materials and methods | 10 |
| Cattle: on arrival feedlot protocol | 10 |
| Trial sampling | 10 |
| Health Check: pen checking protocol and treatment protocol | 10 |
| Results | |
| Medlogic data | 12 |
| Expected results laboratory tests | 13 |
| Conclusion | 14 |
| Discussion | 15 |
| Acknowledgements | 16 |
| References | 17 |

Introduction

Alberta beef production is Canada's largest. About 60 percent of all beef produced in Canada originates from this province. With about 5.5 million head in total and 2.18 million head finished in 2009 it is a large industry.¹ It is clear that a lot of effort is made to run ranches and feedlots as economically profitable as possible. An important part of this is prevention and treatment of sick animals. A better health status decreases costs of treatment, use of medication and lost of income due to a lower growth rate, welfare of animals is less affected and food safety is improved because of a smaller risk of pathogens and residues of medication in food. After transport of calves from the auction mart to the feedlot, calves are processed at the feedlot. This means they get vaccinated, earmarked, branded and given a shot of preventive metaphylactic antibiotics. When the calves are settled in their lot, they are checked every day and pulled out of the pen when treatment is needed. The checking is done by humans on foot or riding horses. Their observational qualities are a golden standard for disease detection. However, humans are always a very variable and expensive factor in a system so disease detection based on actual facts of the animals is interesting. If it would be possible to detect disease based on more objective facts, it would be profitable for many of the reasons listed above.

Feedlot system

Fall placed calves in feedlots can have different origins. The calves are typically born in the spring so when they arrive at the feedlot they are around half a year of age. Alberta does not have enough cow-calf herd capacity to provide enough animals to fill up the feedlots. Cow-calf herds are often smaller herds, the average herd size is 150 cows, in areas higher up in the mountains with an extensive way of management. Feedlots capacity ranges from a few hundred to 40,000 head at one time. About 100 feedlots with capacities over 1,000 head produce at least 75 per cent of the finished beef cattle in Alberta though. To provide enough head to fill up the feedlots, calves are imported from other provinces as well. Saskatchewan and Manitoba are main suppliers of calves. Long transportation and mixing of calves at auction marts improves the risk of respiratory diseases during the first weeks at the feedyards.² Another source of calves are the dairy herds in Alberta. A small percentage of the surplus of bull and heifer calves goes to feedlots to be fed. Holstein meat is especially popular in authorities like the United States army because of the homogeneity of the meat which makes sure that every meal is similar to another.

Calves are normally fed until a live weight of 1200 to 1400 lbs, which is around 600 kilograms. Cattle are often grass pasture fed in the cow-calf herds. In the feedlots however, they are grain fed. The percentage of grain rises the longer the cattle are in the feedlot. This high amount of grain in the feed is the main cause of metabolic disorders like ruminal acidosis, bloat and laminitis, some of the major health issues at a later age.

Research project

The main research project that provided my internship is a collaboration of different parties. It works under the title of: 'Developing and evaluating an automated diagnostic method for feedlot cattle' and is part of a larger study with University of Alberta. A company called Growsafe Systems Ltd. developed systems to measure feed and water intake in a feedlot setting. Calves are recognized by their ear tags when they enter the feed bunks or the drinking system. The precise amount of feed and water intake is measured by the equipment based on weight of the food and flow meters for the water. During drinking, calves have to step up a platform with their front legs to reach the water bowl. This platform measures the front weight of the calf and converts that into an estimation for the total body weight. This way it is

very easy to follow the growth of calves as well. Growsafe Systems Ltd. is doing research whether or not the behavioural measurements can predict disease. It would save a lot of effort for the cowboys if they only have to pull those animals that are recognized by the equipment instead of making their own diagnoses and observations. University of Calgary supports Growsafe's research in determining the actual health status of the calves on arrival and when they are pulled. Blood results are considered as a golden standard to see if animals are actually sick when they are pulled. This can confirm the Growsafe and cowboy diagnostic methods. University of Calgary researchers also look at the differences in immune status on arrival and compare the animals that do and do not get sick. There might be a predictive value in this immune status which can help determine the chance of calves getting sick or not. Cortisol levels in hair samples are tested both on arrival and on close out. This way it is possible to see the stress levels in the animals before entering the feedlot and during their stay at the feedlot. University of Alberta participates in this project by comparing gene expression in sick and healthy cattle with their initial gene expression on arrival in the feedlots. If it would be possible to find any genetic differences between animals that get sick and animals that do not, it could be used in future breeding choices. The trial runs at two different feedyards. The first is Morison farms located in Airdrie, the second is Kasko Cattle Company located in Coaldale. Also the local veterinary clinics are participating in this project by taking blood samples, arranging logistics and doing special post mortems on the mortality causes. Veterinary Agri Health Services is the clinic situated in Airdrie and Coaldale veterinary clinic works together with Kasko Cattle Company.

BRD

Bovine Respiratory Disease is a term used for different respiratory diseases. Another common name for this syndrome is 'shipping fever' after the fact that calves are mostly affected after transport from their original farm to feedlots. BRD is the largest health problem at feedlots with the most economical impact. BRD can be caused by numerous different bacterial and viral agents. A perception often made is that the damage of the respiratory tract by viral agents paves the road for bacterial infections. Examples of viral BRD causing agents are: Para-influenza virus 3 (PI3), Infectious Bovine Rhinotracheitis (IBR), Bovine Respiratory and Syncytial Virus (BRSV), Bovine Viral Diarrhea (BVD). Bacterial agents can be: *Mannheimia hemolytica* and *Pasteurella multocida*, *Haemophilus somnus* and *Mycoplasma bovis*.³⁴

Research internship

My tasks in this project have been twofold. The first part was a literature search for potential blood parameters for the lab confirmation of sick or not sick. This also included the finding and contracting of a suitable commercial laboratory to do the testing. The second part consisted of the comparison between the laboratory and cowboy diagnostic methods. Cowboys select sick animals based on their appearance and the body temperature taken when the calf is ran through the treatment chute. Combined with the treatment history and - protocols individuals were treated. Laboratory diagnostics can be used as a golden standard for determining a diagnosis sick or not sick. It can either confirm the cowboy diagnosis or deny it. By taking blood samples of all the pulled animals a good comparison can be made between several values. Body temperature is an easy measurable one, but also the appearance of the animal expressed in codes can be compared to lab values.

Selection of laboratory parameters

Disease caused by infectious agents (BRD)

Inflammation in general causes changes in blood values. It is however, hard to predict what those changes exactly will be.

Changes in Complete Blood Count (CBC) depend on many different factors, such as the properties of the infectious agent, age of the individual animal and status of the inflammation (acute to chronic). Therefore, a combination of laboratory parameters is used to determine if an animal is diseased or not and interpretation of laboratory results must be done very carefully.

White blood count: Neutrophilia is often seen in acute inflammations accompanied by a left shift. When the inflammation becomes more chronic, the left shift diminishes. Cattle however, have a small storage pool of segmented neutrophils in bone marrow, therefore it is common for cattle with an acute bacterial infection to have a neutropenia. Increased neutrophil production, however, can result in a neutrophilia within days. Lymphopenia is common in acute inflammatory response too because lymphocytes move from the blood into inflamed tissue. Lymphocytosis is seen in chronic infections. Monocytosis is commonly seen when necrosis occurs, that can happen early or late in inflammatory responses but is normally seen in chronic infections. Eosinophilia is seen in animals with atypical interstitial pneumonia and acute pulmonary emphysema.

Red blood cell count: Slight anemia is seen in inflammations caused by bacterial infections and origins in a decreased erythrocyte survival and production due to chronic infection. Also thrombocytopenia can occur because of decreased platelet survival or production during bacterial infections.

Protein counts: Hyperproteinemia can occur during acute infections, but is seen also in animals with ruminal acidosis and sepsis. Hyperglobulinemia is typically seen during chronic pneumonia. Hyperfibrinogenemia is often seen during pneumonia but is a very contradictory factor. In literature, completely opposite statements on this subject are posed. Berry (2004) states that Fb is not reliably associated with BRD although Nikunen (2006) concluded that Fb is useful in this case.^{5, 66784}

Based on literature it is expected that the average outcomes of these blood values of all pulled calves together show a typical acute inflammatory reaction. That means a neutropenia, lymphopenia, high total protein and fibrinogen and some abnormalities in red blood count.

Disease caused by non infectious reasons (ruminal acidosis)

Metabolic disturbances are the most important non infectious health problems in feedlot cattle. After BRD, they cause most mortality and morbidity. Especially acute and subacute acidosis is of great interest; cattle are fed on a high grain diet and the abrupt changes in diet are hard to cope with for the bovine rumen. Diagnosis of acidosis is very challenging in a feedlot setting though. Acute acidosis may be recognized by the clinical signs an animal can show; incoordination, lethargy, anorexia, diarrhea, lameness etcetera. Cattle with subacute acidosis however, hardly show any clinical signs and are thus not recognized by feedlot crew. A lot of research has been done on the subject of finding proper parameters to detect acidosis. Ruminal pH was thought to be useful to measure. However, studies have shown that the fluctuation in time is so great that measuring the pH through rumenocentesis (percutaneous needle aspiration) at one point in time in a single animal does not have any diagnostic value. In a dairy setting ruminal pH could be used to monitor the entire herd health for subacute acidosis. In this case a minimum of 30% of a high risk group of at least ten cows should show a decreased ruminal pH.⁹ A Danish study, however, shows that the predictive value of ruminal pH for acidosis is very low, neither do blood values (lactate dehydrogenase, β -

hydroxybutyrate and fructosamine.¹⁰ Other blood values that are shown to change during acidosis are: blood pH, D-lactic acid, bicarbonate, base status, packed cell volume, endotoxins and inflammatory mediators. Only the base excess status is a really clear marker of subacute acidosis, all other parameters only deviate very slightly to their physiological value. These parameters might be extended with the measuring of pO₂, pCO₂ and other acid-base blood values.⁹ The biggest challenge in our study was the fact that it took normally 12-36 hours to get the samples to a commercial lab. Also, the temperature the samples were exposed to could not always be standardized considering winter conditions. It is described that time delay and storage temperature of bovine venous blood has a large effect on blood gas and acid-base values. Already after one hour, Base Excess and Bicarbonate values were significantly different from the base line testing.¹¹ It is understandable that ruminal pH and the formerly described blood values were not of any value in our feedlot setting.

A study performed by the University of Manitoba shows that experimentally induced subacute ruminal acidosis causes a systemic inflammatory response because of a rise of Lipopolysaccharides in the rumen. LPS translocates across the ruminal epithelium into the systemic circulation where it causes inflammation. This is an indication for the possibility to diagnose ruminal acidosis with inflammatory parameters. Acute phase proteins in acidotic cattle were significantly higher than the levels in hay fed cows. Both Haptoglobin and Serum Amyloid-A were found to be useful blood parameters in the recognition of acute and subacute ruminal acidosis.¹²

Acute Phase Proteins

During any kind of infection the immune system is activated. Traditional ways of testing the immune system can be: complete blood counts, white blood cell differentials, platelet count, fibrinogen and total protein. Because all the parameters spoken of above show large varieties between animals both in BRD and ruminal acidosis it is worth it to extend the search for good parameters. Acute Phase Proteins (APP's) are used more and more. APP's are a variety of proteins that rise during the first hours or days of an infection as an effect of the innate immune system. They have a low specificity for particular diseases but are highly sensitive for the presence of pathological lesions which makes them suitable for general health monitoring. In this study they are very useful because the reason for disease was less important than the diagnosis sick or not sick. APP's typically react to all sorts of inflammation. Not only agent borne inflammations but traumas cause an APP reaction too. It is even described that stress due to long road transport can cause a rise in APP's.

The reaction of APP's differs between species. Some proteins react a lot stronger than others. APP's are highly species specific (Table 1).¹³

| Species | Major APP | Moderate APP |
|----------------|------------------|---------------------|
| Cat | SAA | AGP, Hp |
| Dog | CRP, SAA | Hp, AGP, |
| Horse | SAA | Hp, |
| Cow | Hp, SAA | AGP |
| Pig | CRP, MAP, SAA | Hp, |

Examples of APP's are: SAA: Serum Amyloid A, CRP: C Reactive Protein, Hp: Haptoglobin, AGP: α-1 Acid Glycoprotein, Cp: Ceruloplasmin, Fb: Fibrinogen.

Major, moderate and minor APP's

To be a useful parameter for disease in a specific animal species, APP's have to meet a number of requirements. Based on these requirements APP's are divided in major-, moderate- and minor APP's which can differ totally per species. A major APP has a low serum concentration in healthy animals. Whenever the animal experiences disease, the serum concentration rises a 100- or 1000 fold the basic concentration. There will be a peak concentration after 24-48 hours after the initial stimulation and the concentration will decrease fast when the inflammatory reaction stops. A moderate APP will only have a rise in serum concentration of 5-10 times the basic concentration. The peak shows of after 2-3 days and the concentration decrease is slower. A minor APP only elevates the serum concentration with 50-100%. It is even possible to have a negative APP, where the serum concentration drops during inflammatory reactions. There are little scientific publications on that topic though.

It is fairly clear that major APP's are preferable in terms of sensitivity over moderate or minor APP's.¹³

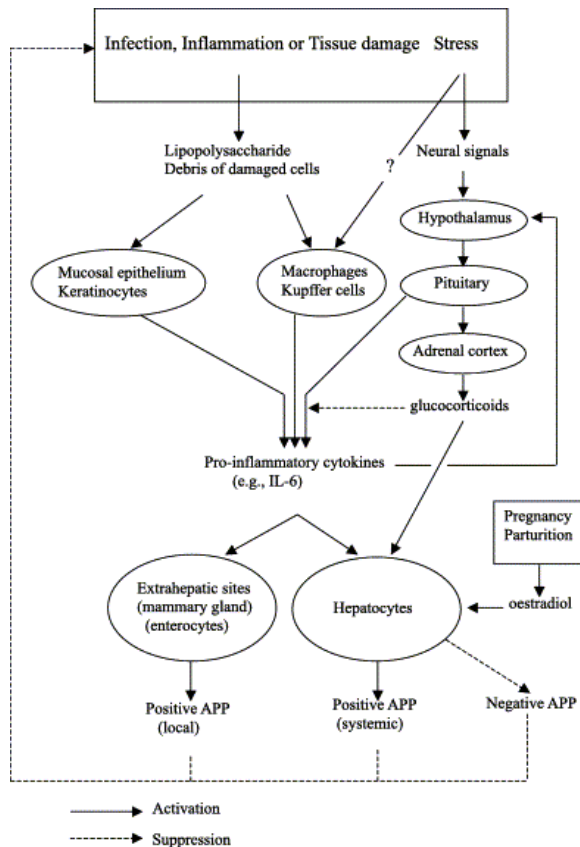
Functions of APP's

Acute Phase Proteins are part of the complete inflammatory reaction. Different APP's have different functions. It is beyond the scope of this report to discuss the functions of all APP's. Haptoglobin will be discussed in more detail however, since this is the most important bovine APP.

In general Haptoglobin (Hp) helps to return to homeostasis. It inhibits the inflammatory reaction by binding to free Haemoglobin (Hb), which is toxic and pro-inflammatory, in the plasma and reduces the oxidative damage associated with haemolysis. Also Hp has an inhibitory effect on granulocyte chemotaxis, phagocytosis and bactericidal activity. Besides this, Hp may inhibit mast cell proliferation, prevent spontaneous maturation of epidermal Langerhans cells (the antigen-presenting cells of the skin) or suppress T-cell proliferation.

Bovine APP's

In the case of bovine medicine, Haptoglobin (Hp) and Serum Amyloid A (SAA) are major APP's. α -1 Acid Glycoprotein is a moderate APP. Hp is mostly used in bovine diagnostics. SAA would be a good one too, except for the fact that lab testing of SAA is much more difficult and expensive than testing Hp. Also SAA reacts less good to respiratory disease than Hp does. Healthy cattle present a basic serum Hp concentration below 20mg/liter. After stimulation the concentration can rise over 2g/liter within a couple of days of infection. Hp is proved to be useful in cases of mastitis, enteritis, peritonitis, pneumonia, endocarditis, endometritis etcetera (Table 2).¹³



Pro-inflammatory cytokines activate the hepatic acute phase response. Especially interleukin-1, interleukin-6 and tumour necrosis factor α are important.

Figure 1. Pathways for the activation of APP's.¹⁴

| Table 2. Bovine Diseases where an Acute Phase Response has been Described. ³ | |
|---|---|
| Acute Phase Protein | Disease/Condition |
| Haptoglobin | Mannheimia haemolytica Pasteurella multocida Bovine viral diarrhoea virus Bovine respiratory syncytial virus Foot and mouth disease virus Mastitis Clinical respiratory tract disease Castration Metritis Uterine bacterial contamination Hepatic lipidosis |
| SAA | Mastitis Mannheimia haemolytica Bovine viral diarrhoea virus Bovine respiratory syncytial virus |
| AGP | Hepatic abscesses Metritis Mastitis Respiratory tract disease |

APP's are not influenced by the diet that is fed or by time after feeding.⁶ There are indications that Hp is a clear indicator for ruminal acidosis as well. Lipopolysaccharides in the rumen rise and enter the blood. They cause an inflammatory reaction and that causes APP's to rise. More research is needed at this point. If this would work, Hp would be a very useful indicator for disease in feedlots. It would cover BRD, ruminal acidosis and almost all other inflammations. Hp is promising to be used for testing animals just before slaughter as well. Animals with elevated levels are controlled extra to improve food safety. It is shown that there is a 6 to 40 fold increase in Hp levels in animals with infectious, metabolic or traumatic disease at slaughter.¹⁵

Laboratory Haptoglobin tests

Hp can be tested in two different ways. The more old fashioned test uses the fact that Hp binds to free Haemoglobin (Hb). The total binding capacity of Hb is measured by looking at the peroxidase activity. This activity rises when more Hb is bonded because free Hb is acidic so binds to peroxidase. Binding to Hb is one of the physiological functions of Hp to prevent oxidative damage during infection. This test is being referred to as the colorimetric method, Hp-Hb binding assay or peroxidase activity test. The biggest challenge in the use of this test is the fact that it becomes unreliable when samples are haemolysed. In haemolysed samples, the amount of Hb in the test is not reliable anymore so the binding capacity can not be measured accurately. Also active and inactive forms of Hp, presence of serum peroxidase inhibitors such as cystine and glutathione and the presence of proteins with peroxidase activity in the serum can influence the outcome of the test. Since field samples are never perfect and haemolysis occurs sometimes because of logistical challenges, this is not the ideal test. Also samples taken at meat inspection are often haemolysed so the need for a better test is clear.^{16,17}

A newer way of detecting Hp levels is the use of ELISA tests. One of the ELISA's uses monoclonal antibodies against Hp. This way of testing is a lot more sensitive because it directly measures Hp concentration instead of peroxidase concentrations. Cross reactivity with other serum proteins was measured to be less than 0,1%. Detection limits were found to be 0,344 µg/ml and 1,589 µg/ml for respectively healthy adult male and female cattle which is low compared to a base Hp level of 20 mg/liter (20 µg/ml). A study that compared both the Hp-Hb binding- and the ELISA test on healthy and (experimentally induced respiratory) diseased cattle showed that the ELISA test is much more reliable. ELISA testing of Hp is thus mostly used in field and laboratory trials.¹⁸

Field trial

Materials and Methods

For the entire project, two pens at two feedlots were filled up with cattle during November 2010. At Morison's two pens with 213 animals each and at Kasko's two pens with 160 animals each, were filled up. All pens were equipped with the water and weight measuring systems and one pen at Morison's was equipped with the feed intake measuring bunks as well.

Cattle: on arrival feedlot protocol

Upon arrival animals were processed. They received an injection with oxytetracycline (Tetradure LA[®]) instead of the normally used tulathromycine (Draxxin[®]). There were no antibiotics administered through the food for the whole duration of the trial. Also feedlot specific ear tags were linked to ear tags that are necessary for recognition of the animal by the Growsafe equipment. All information was documented in the Medlogic software program used at the feedlots to keep track of every individual animal. The animals were branded as well, castrating was not necessary since all bought cattle were steers.

Trial Sampling

Besides the regular processing procedures, blood and hair samples were collected. At both feedlots, 75 random hair samples were taken from the jugular area at arrival. At Morison's all the blood samples were taken from the jugular vein. At Kasko's the samples on arrival were taken from the tail vein and the treatment samples from the jugular vein. This was due to a difference in processing and treatment chutes. The processing chute was not appropriate for jugular bleeding because of large, not removable neck bars and the impossibility to tie out the animal properly. On arrival four blood tubes were taken: two red top serum tubes, one purple top EDTA top and one PAX tube. Per feedlot 150 PAX tubes were taken. All the tubes were kept cool until arrival in the lab. Serum tubes were spun down the next day after an overnight stay in the refrigerator, at 2000g for 15 minutes at 20°C. The obtained serum was aliquoted in maximum six one millilitre vials. After aliquoting they were frozen at -20°C. The EDTA tubes were frozen (-20°C) after arrival in the lab. The PAX tubes were frozen after incubation at room temperature for 2-72 hours in the -20°C freezer. Serum will be used for determination of immune status. EDTA and PAX tubes will be used for determination of gene expression. For a period of three to four weeks after arrival of the animals blood was taken from each pulled animal. Only first pulls were sampled with the exception of first pulls being a secondary reason, footrot or joint infection for example, and the second pull was for BRD. Four blood vials were taken. Two serum tubes, one EDTA tube and one PAX tube. The PAX tubes were used for determination of gene expression in the sick animals. The EDTA tubes were used for direct CBC's, white blood cell differentials and platelet count. Serum tubes were used for direct testing on fibrinogen and total protein and frozen for later testing on immune status and acute phase proteins. Samples were sent to a commercial lab at least within 48 hours so degradation of blood was not an issue.

Health Check: pen checking protocol and treatment protocol

Pen checking was done by employees of both feedlots. When running the animals through the chute codes were written down in the comment boxes in Medlogic. The following codes were used: code D for drooped head/ears, code E for appearance of Eyes: crusted, glassy, dull, sunken, code C for Crusted muzzle, nasal discharge, code R for Reluctance to move, code G for Gaunt appearance. Also the initials of the pen checker were filled in so differences in

checking strategies can be looked into as well. As the cut of point for treatment, a temperature of 104,00°F (40°C) was used. Animals were considered sick and were treated if their temperature exceeded 104°F. However, this strategy was not held onto very tightly because the appearance of the animals pointed towards sickness so clearly sometimes that they were treated even if the temperature was not high. These animals were saved in Medlogic as 'No fever treatment'. Sick animals were treated according to the standard operating procedures written by the local veterinary clinics. This means that first pulls for BRD were treated with Baytril® (enrofloxacin) and second pulls for BRD were treated with Nuflor® (florfenicol). If animals remained diseased after treatment with these drugs, other medication could be used. Animals were moved to the chronic pen if other drugs than Baytril® or Nuflor® had to be used though, except when the indication was another kind of disease like a joint infection. A move to the chronic pen meant that the animals were excluded from the trial as feed and water intake data could no longer be collected.

Results

Medlogic data

Because of the fact that the study was not finished by the time this report was written, only limited information was available for analysis. The next paragraph shows an overview of the Medlogic data of Morison's two pens. This information was obtained November 24th 2010 and covers an estimated ninety percent of the first pulls in pen 2 and eighty percent of the pen 9 first pulls. It is not useful to analyze the re-pulls at this point because the pull rates were still high so information would be very incomplete.

First pulls until Nov 24th

| | |
|-------------------------|--------------|
| Pen 2 (I1) Black | 157/213: 74% |
| Pen 9 (I2) Grey | 147/214: 69% |

Diagnoses pen 2

| | n | Avg Temperature (°F) | Avg Weight (pounds) |
|---------------------------|----------|-----------------------------|----------------------------|
| BRD | 98 | 105,10 | 663 |
| No treatment | 30 | 102,64 | 635 |
| No fever treatment | 29 | 102,76 | 667 |
| Footrot | 0 | - | - |
| Total | 157 | | |

Diagnoses pen 9

| | n | Avg Temperature (°F) | Avg Weight (pounds) |
|---------------------------|----------|-----------------------------|----------------------------|
| BRD | 85 | 104,95 | 723 |
| No treatment | 8 | 101,73 | 688 |
| No fever treatment | 52 | 102,94 | 711 |
| Footrot | 2 | 101,25 | 651 |
| Total | 147 | | |

Average weight at first pull

| | |
|-------------------------|------------|
| Pen 2 (I1) Black | 658 pounds |
| Pen 9 (I2) Grey | 716 pounds |

This data shows results that were pretty much expected except for the extraordinary pull rates. Pulled animals placed in the 'BRD' category had high temperatures and clear clinical signs of disease. Animals placed in the 'no fever treatment' category had clinical signs but did not show a high temperature, because their appearance they were treated anyway. 'No treatment' animals were pulled because of clinical signs but did not show a temperature. While taking them through the chute they did not look bad enough to treat them so they were released to their pen again. It is interesting to see that the weight of 'no treatment' animals both in pen 2 and pen 9 was about twenty to thirty pounds less than the weight of 'BRD' and 'no fever treatment' animals. This could be caused by the fact that lighter animals might be pulled more often than heavy animals because they might look worse than the rest of the pen just because they are smaller or thinner. A second point of interest in this data is the fact that the distribution of the pulled animals among the three categories is different in pen 9 than it is in pen 2. Pen 9 has a lot less 'no treatments' than pen 2; 8 versus 30. On the contrary, 'no fever treatments' are a lot higher in pen 9; 52 versus 29. The animals in pen 9 arrived about two weeks after pen 2. This might be a 'cowboy bias'; because of the high morbidity in the cattle

they were rather treated than put back in their pen without treatment when all the effort of pulling an animal was done already. It is also possible that the reason for this is the weather since there was a very cold period just after pen 9 animals arrived.

Expected results laboratory tests

All samples were tested on CBC's, white blood cell differentials, platelet count, fibrinogen and total protein. Hp is still to be tested.

Based on literature it is expected that the average outcomes of these blood values of all pulled calves together show a typical acute inflammatory reaction. That means a neutropenia, lymphopenia, high total protein and fibrinogen and some abnormalities in red blood count. In this study, however, the goal is to determine if an individual animal is sick or not. Because the individual variation between animals is so large, there will be many animals that were diagnosed diseased by the cowboys that do not show a typical inflammatory blood count. To help determining the diagnosis, Hp will be very valuable because of its proven usefulness in feedlot BRD cases and ruminal acidosis as well. It is expected that Hp will be used as the determinant whether an animal is diseased or not.

The correlation between laboratory diagnosed sick animals and pulls in the BRD group is expected to be high. Those cattle had a diseased appearance and a high temperature which leaves little doubt for the presence of disease. The 'no fever treatment' and 'no treatment' cattle are a lot more interesting to compare to laboratory outcomes. A large part of the 'no fever treatments' will be diagnosed positive by laboratory results too, temperature is not a hundred percent accurate parameter so there will be sick animals without high temperatures in that category. The 'no treatment' group will be the most diverse. These cattle were pulled for a reason by the cowboys. While taking them through the chute though, they did not have a temperature and the appearance of the animal was not bad enough to treat it. It is possible that these cattle are in an early state of disease and do not show signs too much. In this case, blood parameters could show disease already. Another possibility in this group is that cattle fool the cowboys and that they are not sick at all. In this case blood parameters would not derive from healthy levels. It is expected that a fairly part of the 'no treatment' cattle do show signs of disease in their blood. This statement is based on the fact that quite many animals were pulled again after a short time and had high temperatures by then (not shown in diagrams).

Conclusion

After studying literature about acute phase proteins in ruminants it can be concluded that Hp is a promising diagnostic value. It is a major acute phase protein that rises significantly during inflammation, infection and trauma. This way Hp can be used as a general marker of acute disease. It is not specific for any organs or inflammations and therefore cannot be used for diagnosing specific diseases. It is shown that Hp has an excellent reaction to respiratory disease and therefore it is very useful in this study because most of the sick cattle had BRD. Also the fact that Hp reacts to subacute ruminal acidosis is very useful in this study because ruminal acidosis remains very difficult to test both in the blood as in the rumen itself. Because the laboratory results were not analyzed yet, there can only be expected conclusions about the comparison of laboratory and cowboy diagnoses. It is expected that there is a relatively high correlation between laboratory diagnoses and the Medlogic data. Especially Hp will have a high correlation with the Medlogic data since it is probably the most reliable parameter for general acute disease. CBC's will show huge variance between animals because the peaks of abnormalities are a lot shorter in time and therefore there is a smaller chance of catching those abnormalities at the right time. The Hp peak lasts at least a few days before the level is back to normal again.

Of all the pulls that are recorded in Medlogic, the 'no treatments' will be the most interesting to compare with laboratory results. These animals were pulled for some reason but appeared to be not sick enough to treat them. Often after a few days these animals were pulled again and showed temperatures by then. It will be interesting to see if there is any sign of disease in the blood tests at the time of first pull already.

Discussion

During field trials there will always be unexpected challenges in logistics and results. In this trial calves were given oxytetracycline (Tetradure LA) on arrival as a preventive metaphylactic drug. This antibiotic is normally applied to winter placed calves and not to fall placed calves who are considered high risk cattle. The effect of this was that the pull rates were excessively high. It is questionable if the artificial pull rates that were created are still comparable with reality. The sickness the animals showed might not be alike the sickness animals show when they are treated with different antibiotics on arrival. The logistics of taking blood samples, processing them in the University lab and transporting them to the commercial lab were challenging too. There were two feedlots at different distances from Calgary and different people were sampling and processing samples. Also the transportation and processing conditions of the samples were not always the same. Some samples were tested the same day, others only the next day. Sampling conditions were subject to outside weather changes; during the trial we experienced temperatures varying between +10°C and -40°C. All arrival and sick animals were sampled from the jugular vein except the arrival samples at Kasko's, they were taken from the tail vein. Of course these varying situations come with field trials, however, standardization of all conditions is ideally aimed for in research projects. Besides logistical differences that can influence the quality of blood samples, it is important to realize that road transport and processing on arrival can have a great impact at inflammatory parameters being very stressful events for cattle. Stress can trigger inflammation and increase Hp levels. Therefore blood results of animals that were pulled in the first three to five days after processing might not be very reliable. Besides sampling, the scale at Morison's farms was not always measuring accurately which might directly influence results and doses calculations of medication. Another point of discussion is the fact that calves were not all processed immediately after arrival at the feedlot. Some truck loads had to wait for one or two days to be processed. In the meantime no behavioural measurements were done and it might have been easier for these animals to get sick since they did only get antibiotics at the point of processing.

Acknowledgements

This study and my own internship could not have been performed without the help of many others. First I would like to thank Doctor Karin Orsel for the good cooperation the both of us had for the three months that I have been at the University of Calgary. Without the participation of Doctor Phil Klassen and the Coaldale Veterinary Clinic and Doctor Craig Dorin and Veterinary Agri Health Service in Airdrie it would never have been possible to perform this whole trial. Also the participating feedlots and their crew are of a great value to this trial. Without their help and patience there would not be any results by now. I would like to thank Alberta Innovates, Biosolutions and Growsafe systems Ltd as well for their participation in funding and supplies for this study. Also Doctor Ruurd Jorritsma is very appreciated for his supervisory work at the University of Utrecht. Last but not least I would like to thank all the wonderful people I met in Calgary and surroundings during my stay who helped me having an awesome time.

References

1. www.albertabeef.org.
2. Cynthia M. Kahn. in *Merck Veterinary Manual* , 2005).
3. David E. Anderson & D. Michael Rings. in *Food Animal Practice, current veterinary therapy* (Saunders Elsevier, 2009).
4. Bradford P. Smith. in *Large animal internal medicine* (Mosby, 2002).
5. Bernard F. Feldman. in *Schalm's veterinary hematology* (Blackwell Publishing, 2006).
6. Berry, B. A. *et al.* Effects of dietary energy and starch concentrations for newly received feedlot calves: II. Acute-phase protein response. *J. Anim. Sci.* **82**, 845-850 (2004).
7. Nikunen, S. *et al.* Association of bovine respiratory disease with clinical status and acute phase proteins in calves. *Comp. Immunol. Microbiol. Infect. Dis.* **30**, 143-151 (2007).
8. Steven L. Stockham & Michael A. Scott. in *Fundamentals of veterinary clinical pathology* , 2008).
9. Nagaraja, T. G. & Lechtenberg, K. F. Acidosis in feedlot cattle. *Vet. Clin. North Am. Food Anim. Pract.* **23**, 333-50, viii-ix (2007).
10. Enemark, J. M., Jorgensen, R. J. & Kristensen, N. B. An evaluation of parameters for the detection of subclinical rumen acidosis in dairy herds. *Vet. Res. Commun.* **28**, 687-709 (2004).
11. Gokce, G., Citil, M., Gunes, V. & Atalan, G. Effect of time delay and storage temperature on blood gas and acid-base values of bovine venous blood. *Res. Vet. Sci.* **76**, 121-127 (2004).
12. Gozho, G. N., Plaizier, J. C., Krause, D. O., Kennedy, A. D. & Wittenberg, K. M. Subacute ruminal acidosis induces ruminal lipopolysaccharide endotoxin release and triggers an inflammatory response. *J. Dairy Sci.* **88**, 1399-1403 (2005).
13. P.D. Eckersall. *Acute Phase Proteins as Biomarkers of Disease in Production Animals*, 2006).
14. Moshage, H. Cytokines and the hepatic acute phase response. *J. Pathol.* **181**, 257-266 (1997).
15. P.D. Eckersall. Acute Phase Protein: Biomarkers of Disease in Cattle and Sheep. *Cattle Practice* **15**, pp. 240-243 (2007).
16. Young, C. R., Eckersall, P. D., Saini, P. K. & Stanker, L. H. Validation of immunoassays for bovine haptoglobin. *Vet. Immunol. Immunopathol.* **49**, 1-13 (1995).
17. McNair, J. *et al.* Evaluation of a competitive immunoassay for the detection of bovine haptoglobin. *Res. Vet. Sci.* **63**, 145-149 (1997).

18. McNair, J., Elliott, C. T. & Mackie, D. P. Development of a sensitive and specific time resolved fluorimetric immunoassay for the bovine acute phase protein haptoglobin (Hp). *J. Immunol. Methods* **184**, 199-205 (1995).