Lethal Heat Stress in Dairy Cattle: Unrecognized, Misdiagnosed, Needs Research

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Introduction

Heat Stress (**HS**) affecting dairy cows is widely recognized in the dairy industry in terms of production and management, but not in terms of mortality or diagnosis of potential lethality. Cattle deaths from HS can be extensive. A high mortality rate occurred in heat events in the US this summer where 2 to 10 thousand cattle died in Kansas feedlots (Chappell, 2022, Guilfoil, 2022, Laborie, 2022). Extreme cattle mortality has occurred in previous years as well, in California (2017) 5-10K, Iowa (1995) 3.75K, and Nebraska (1999) 5K (Osgood, 2017, Lees et al., 2019). Mortality due to HS is also reported internationally (Morignat et al., 2014, Vitali et al., 2015). Clearly HS can kill cows in large numbers, which tends to be recognized only because of the enormous unexpected excess mortality associated with extreme heat events, as opposed to diagnosis of risk or cause of death by veterinarians.

While pathological outcomes of HS can include death (lethality), the pathobiology of lethal heat stress **(LHS)** in bovines is not described in prominent current veterinary textbooks nor research literature. Better awareness and understanding of LHS is needed. Perhaps the major reason literature describing differential diagnostics in bovines does not include LHS is that unlike humans or dogs, which are frequently hospitalized for heat stroke, cattle are not hospitalized during heat events, so there is a deficit of observed pathology indicative of LHS. Differential diagnosis of severe HS is also not considered because it typically presents like a nutritional problem, and veterinarians, producers and nutritionists are generally unaware of the disorder, or its presentation or pathology. Consequently, alternate differential diagnoses are pursued, typically nutritional, such as acidosis, mycotoxicosis, or fatty liver. In less severe heat events HS commonly presents with diarrhea, suggesting a gastrointestinal tract (**GIT**) disorder. Severe HS also can present as “downer cow syndrome” which has multiple possible and differential causes (Grünberg, 2022). These include signs that can be present in LHS, including severe hypokalemia, hypophosphatemia, and systemic illnesses or infections such as toxic mastitis or metritis, and hepatic lipidosis or liver failure. Other signature HS related clinical signs are discussed later. However, its initial presentation often suggests that the underlying problem is nutritional.

Additional reasons contribute to lack of awareness of LHS. It is not a recognized pathology in cattle, whereas heat stroke is widely recognized in dogs and humans. There are no pathognomonic signs (i.e., no definitive indicators or diagnostics exclusive to LHS). Unlike LHS occurring with major heat events, it can occur in a low number of cases in a herd with less severe heat / humidity exposure. Important risk factors for LHS, including the intensity and duration of heat and humidity, and whether the exposure is abrupt or cattle are acclimatized, are often not considered.

Ultimately, failure to recognize LHS occurs because it is a complex disorder that is the cumulative outcome of numerous physiological processes and pathogenic pathways that are incompletely understood in human and veterinary medicine. Recognition occurs when a heat event is overwhelmingly obvious, such as the Kansas feedlot deaths, June 2022 (Chappell, 2022, Guilfoil, 2022, Janousek, 2022). However, the veterinarians attending those feedlots described a diagnosis based on the atypical abrupt heat and humidity conditions immediately prior to those deaths, and not so much on understanding specific pathologies of death by heat. However, HS severe enough to be lethal also occurs in dairy cows, but is essentially unrecognized.

The authors have been involved in a number of cases of LHS which were puzzling and misdiagnosed by the veterinarians and nutritionists involved. Four of the cases, described below, provide insight into the pathology and diagnostic challenge of LHS on dairy farms. Each demonstrated different factors that contribute to LHS and illustrate the lack of awareness of LHS by dairymen, veterinarians, and nutritionists.

Case 1:

A dairy milking over 1,000 cows in Idaho had multiple cases of diarrhea, down cows in established lactation, and cow deaths. The dry lot dairy had eleven corrals but only one corral had a shade. The herd veterinarian and the nutritionist made presumptive diagnoses of acidosis. Diets were revised even though the source of “acidosis” was not clear; rations had adequate fiber and were not excessively fermentable. Diagnostics included necropsies by two veterinarians and multiple clinical chemistries. Observations of hyperemia, petechia, ecchymosis, and fibrin at necropsy revealed severe systemic inflammation and coagulopathy. During one of the necropsies the dairyman asked the veterinarian to examine the family dog, which had just collapsed on the lawn. The veterinarian made a presumptive diagnosis of heat stroke, and advised taking the dog immediately to a clinic for cooling, fluids, and electrolyte support. The dog died at the clinic; cause diagnosed as heat stroke. At the time, the veterinarians, nutritionist, and dairyman made no association of the dog with cow deaths, which had numbered approximately 20 in the previous several weeks. No diagnosis for the cow deaths was determined.

Case 2:

A dairy near case 1 had cows with similar presentations, including oddly colored diarrhea, recumbent cows in established lactation, and cow deaths. Also a dry lot dairy, all of the pens had shades. Although this dairy had nearly three thousand cows, it had far fewer cow deaths. Again, necropsies were done but the health problems and cow deaths remained undiagnosed.

On both Idaho dairies the deaths stopped after a relatively short period of time. In spite of the efforts of four experienced veterinarians no definitive diagnosis was made at either dairy. The difference in death rate likely reflects the difference in solar radiation

exposure (shades). The authors later attended a conference where Dr. Lance Baumgard described “Leaky Gut,” a condition initiated during severe heat exposure which causes the gastrointestinal tract (GIT) epithelium to become permeable and “leak” it’s content, including endotoxin. The condition Dr. Baumgard described as heat stress seemed to fit as a reasonable explanation of the HS events at the Idaho dairies.

Case 3:

Several years later (2018) an excellent nutritionist in the Northeast had a herd (~350 cows) that experienced down cows in established lactation accompanied by deaths from mid-June to early July. For each of 4 weeks the author was consulted by the nutritionist about interpretation and plausibility of diagnostics and differentials being proposed. These included clinical chemistries (results were inconclusive), rumenocentesis to assess acidosis risk, liver biopsies and mycotoxin analyses. In the fifth week the nutritionist commented that “The cows might just be too darn hot!”. “Aha!” declared the consultant. The dairy’s freestall barn had a high ceiling, was located on a breezy knoll and had good natural ventilation. The dairy had installed an automated milking system the previous year. The change included construction of “robot rooms” which completely closed in the sidewalls of all four quadrants of the barn, which also had insufficient fans and no sprinklers. The conclusion was that LHS was responsible for unexplained cow deaths based on two contributing risk factors. 1. The closed sidewalls and paucity of fans left the barn poorly ventilated with little heat abatement. 2. The weather had been very cool in May and early June, but in mid-June it suddenly turned very hot, with temperatures in the high nineties (^{o}F) and high humidity. The cows were not acclimatized and adapted to the heat.

Case 4

The fourth case occurred midsummer in Northern New England, again on a dairy that experienced relatively cool conditions in early summer followed by sudden high temperatures for several days. Several cows died; initially necropsies and clinical chemistries did not suggest a diagnosis. However, this dairy had a serious water problem, in that congested water system pipes did not always provide adequate water for the cows. Also, access to waterers was poor. In this case the histories, clinical chemistry, and necropsies early on were helpful in suggesting LHS as the putative cause of the deaths, and possibly limited the number of deaths. In both of these Northeast dairies the lack of acclimatization was a contributing factor, and was exacerbated by ventilation and heat abatement on one dairy, and inadequate water access and dehydration on the other. Learning the history and context was critical in arriving at a diagnosis at both dairies; diagnostics alone were insufficient.

These four cases are important because they reflect that LHS and its pathology are not recognized within the dairy industry. Necropsies, clinical chemistries and other diagnostics were performed in these cases. Knowledge was lacking about how the results, coupled with the environmental conditions, suggested LHS was occurring. These cow death events and the lack of awareness that LHS could be a differential diagnosis

stimulated the initiative to describe the putative pathology and pathogenesis of LHS. The dairies chronicled here were well managed, which suggests that many dairies experience LHS deaths and do not recognize it. Dairy advisors and producers are not familiar with the environmental risk factors, presentation and pathogenesis of LHS, or its potential lethality. Popular press and scientific literature address HS consequences related to productivity and reproduction and management actions like heat abatement, nutritional strategies or additives. There is information about why HS events may be a reason cows die in a herd.

The primary objective of this paper is to increase awareness of LHS in cattle for veterinarians and nutritionists (dairy and beef). The second objective is to contribute to basic understanding of its presentation, recognition, and pathogenesis. There are no detailed published works about LHS or its diagnosis in cattle and more research is needed.

Pathogenesis of LHS

The pathogenesis of LHS is more likely to occur under specific conditions, including high ambient temperatures concurrent with high humidity, poorly ventilated micro environments, exposure to direct solar radiation, and sustained periods without nighttime cooling. The onset of severe HS is also a function of acclimatization status; cows in warmer environments that are gradually exposed to a heat load become acclimatized physiologically whereas cows not gradually exposed do not become heat adapted. This is why in Northern temperate regions in the US, cows, like humans, can be unexpectedly affected by LHS (cows) or heat stroke (people, dogs) (Kadzere et al., 2002, Nienaber and Hahn, 2007, Leon and Kenefick, 2011). Cows that are not acclimatized are at higher risk of LHS pathologies when suddenly exposed to a high heat and humidity. This is especially the case in extreme heat midsummer when the spring and early summer have been cool. Other factors can increase risk of LHS in cattle, such as fatness, high production and dry matter intake, breed, genetics, coat color, overcrowded facilities, preexisting health conditions, extent and efficacy of heat abatement, and hydration status (Brown-Brandl et al., 2006, Sullivan and Mader, 2018). These factors are well known, with published research describing their effects so they are not addressed in this paper. The origins of HS tissue and organ damage that become lethal involve multiple complex systems, including cardiac, vascular, immune, hemostatic, metabolic, redox, renal, respiratory, hepatic and other systems. The complex pathology of severe HS in bovines has not been described, but appears to be similar to and substantially conserved across other mammalian species. Here LHS is described based on research on heat stroke in other mammalian species. The label LHS is used instead of heat stroke because heat stroke includes neurological pathology, which has not been reported in cattle.

The basic pathogenesis of LHS can be described as a four stage progression:

- **Stage 1.** Heat exposure above thermoneutral and the corresponding behavioral and acclimatization responses.
- **Stage 2.** Prolonged severe heat exposure resulting in physiological dysfunction.

- **Stage 3.** Physiological dysregulation and systemic degeneration including concurrent counteracting pro- and anti- inflammatory, oxidative, hemostatic system responses.
- **Stage 4. Lethality** arising from organ dysfunction, respiratory dysfunction, and septic shock.

LHS Pathogenesis Stage 1: Behavioral and Evaporative Cooling Responses

Stage 1 of HS pathogenesis has two elements: Adaptive behavior and evaporative cooling. The stage 1 elements are primarily HS responses, not pathologies, although as heat load severity increases, they contribute to pathologies of stage 2.

Adaptive behavior:

Adaptive changes include reduced dry matter intake (reduces metabolic heat production), changes in meal patterns (eating less during hotter daytime hours and more during cooler nighttime hours), increased time standing, increased bunching, and increased water intake (Burhans et al., 2022).

Evaporative Cooling:

Evaporative cooling exposes body water containing body heat energy (sweat or exhaled air) to cooler drier air where it diffuses into the air and disperses heat energy as it changes from liquid to gas phase (water vapor). Bovine evaporative cooling has three mechanisms: 1) sweating, 2) increased respiration rate (**RR**), and 3) peripheral cooling associated with blood flow redistribution. Sweating is stimulated by elevated skin temperature and provides evaporative cooling (Gebremedhin et al., 2010); sweating also causes loss of potassium in sweat (Kadzere et al., 2002). To a lesser extent, sweating increases sodium loss by renal excretion due to reduced aldosterone level, which is a mechanism to conserve potassium from renal excretion. Evaporative cooling also occurs from increased RR (Robertshaw, 2006, White, 2006). As the heat load intensifies, RR progresses to panting and hypersalivation, which result in salivary loss by drooling, exacerbating loss of sodium and potassium. Panting and hyperventilation cause respiratory alkalosis as increased CO_2 is expired and blood CO_2 levels decrease; respiratory alkalosis stimulates renal excretion of bicarbonate (**HCO_3^-**) resulting in compensatory metabolic acidosis. Overall, the elevated RR causes an alternating acid/base disturbance with alkalosis in the hotter daytime hours and metabolic acidosis in the cooler nighttime. Increased core body and skin temperature stimulate redistribution of blood flow from the core to the periphery to achieve peripheral cooling (Lambert et al., 2002, Cronje, 2005, Wang et al., 2011, Baumgard and Rhoads, 2013). This redistribution involves vasodilation of peripheral vasculature, accompanied by vasoconstriction of the gastrointestinal tract (**GIT**) to maintain systemic blood pressure. These stage 1 mechanisms have been extensively researched and are relatively well recognized and understood in cattle.

LHS Pathogenesis Stage 2: Prolonged Severe Heat, Physiological Dysfunction

When heat load is severe and prolonged, cows become hyperthermic which progresses to physiological dysfunctions, sequelae to stage 1 adaptive heat responses (blood flow redistribution, acid/base disturbances, and electrolyte derangement).

GIT Permeability:

The most consequential dysfunction sequela of stage 1 HS is the development of GIT hyperpermeability, colloquially termed “leaky gut” (Wang et al., 2011, Baumgard and Rhoads, 2013, Koch et al., 2019). While the occurrence of GIT hyperpermeability has been clearly demonstrated in many mammalian species, the primary causal mechanism and specific location of GIT “leakiness” in ruminants is not known definitively (Burhans et al., 2022). Multiple causal mechanisms for GIT hyperpermeability have been suggested, including heat exposure alone (Dokladny et al., 2006), thermal damage to tissues, hypoxia (inadequate blood oxygen) due to hypoperfusion (Salzman et al., 1994), oxidative and nitrosative stress, epithelial damage due to hyper-osmolality and cell swelling, ruminal histamine, splanchnic mast cell activation, GIT endotoxins, and mast cell secretions such as proteases and histamine, increased cortisol, and tissue acidosis (Burhans et al., 2022). Rumen pH decrease might also contribute; pH appears to vary from normal during HS, although the variability, diurnal pattern, range, or duration of rumen pH changes during HS has not been well investigated nor definitively profiled (Burhans et al., 2022). Reduced rumen motility (Attebery and Johnson, 1969) might contribute to pH decrease during HS.

Endotoxin Translocation:

Hyperpermeability of the GIT during HS facilitates translocation of endotoxins out of the GIT into systemic circulation. Endotoxins (lipopolysaccharide, i.e., **LPS**) are the “structural parts of gram negative bacteria cell wall, are potent immune stimulating antigens, and are comprised of three major regions: a side chain, core polysaccharides, and lipid A” (Andersen, 2003). The side chain of repeating units of oligosaccharides differs between Gram-negative bacterial strains and is unique to specific strains. The location of LPS efflux from the GIT in ruminants is not definitively known (Gao et al., 2022). An experiment in goats utilizing a 24 hour exposure to HS (35^{o}C/95^{o}F) concluded that net efflux of LPS occurs from both the intestines and the rumen (Wang et al., 2011), consistent with earlier in vitro work in bovine epithelia (Emmanuel et al., 2007). A small amount of endotoxin escapes the GIT normally; the net amount that appeared in the portal vein during HS increased 228%. The net LPS absorption measured by Wang in the mesenteric vein was only 20% of the net portal vein flux. Thus it appears that most of the efflux is primarily from the rumen, consistent with speculation based on earlier research (Cronje, 2005). The efflux proportions from these locations were the same during thermoneutral conditions, suggesting that location of LPS efflux from the GIT may not change or differ during HS.

Rumen pH:

Panting, open mouth panting, drooling and saliva loss increase as heat intensity and duration of exposure increase. Saliva loss results in Na^+ and bicarbonate (**HCO_3^-**) loss, concurrent renal excretion of both Na^+ and HCO_3^- increases these losses, presumably decreasing supply (the authors are unaware of studies which have quantified this). Both Na^+ and HCO_3^- are needed for VFA absorption from the rumen lumen; compromised supply would presumably reduce VFA absorption out of the rumen and reduce ruminal buffering (Aschenbach et al., 2011, Burhans et al., 2022). Saliva loss from drooling and renal HCO_3^- excretion during respiratory alkalosis could potentially result in reduced rumen pH. As noted above, this remains plausible but has not been investigated.

Permeability & Rumen pH:

Two things occur in the rumen when rumen pH is reduced during acidosis. First, there is an increase in ruminal endotoxin content due to high starch fermentability or low effective fiber, (Gozho et al., 2005, Li et al., 2012). An increase in colonic endotoxin occurs when hind gut acidosis results from high colonic starch loads. Second, it is known that there is an increase in ruminal histamine production with greater ruminal fermentability (Sanford, 1963, Garner et al., 2002); higher fermentability decreases rumen pH. Increasing histamine flux from the rumen occurs at lower pH (Plaizier et al., 2008), likely by stimulating epithelial inflammation (Sun et al., 2017) or facilitated by low rumen pH (Aschenbach and Gäbel, 2000). If and how these consequences of increased RR, panting and drooling contribute to HS effects is not definitely known, but potentially they could contribute to the development of hyperpermeability of the GIT epithelium; research is needed.

Hepatic Overload:

Endotoxin and intact bacteria translocated from the GIT are conveyed by the portal vein to the liver where they are degraded by Kupfer cells. Kupfer cells are hepatic macrophages. Activated by endotoxin and bacteria they release cytokines (immune system proteins providing immune regulation and communication), prostanoides (inflammation mediators), nitric oxide and reactive oxygen species (pro-oxidants) that degrade bacteria and detoxify LPS (Bilzer et al., 2006, Dixon et al., 2013). Normally the liver is presented with only a small amount of “leaked” LPS and detoxifies it. But as the GIT epithelium becomes increasingly permeable the efflux delivered to the liver increases and can overwhelm the capacity of the Kupfer cells, and then the flux of endotoxin becomes systemic (Wang et al., 2013).

Renal Dysfunction & Tubular Necrosis:

Kidney damage is common during heat stroke and LHS. Causes include core body hypoperfusion due to blood flow redistribution, although given the retroperitoneal locations of the kidneys direct thermal damage might also be a factor. Damage can also be caused by myoglobin exposure resulting from myofibrilar (muscle) protein degradation

(Bruchim et al., 2006, Leon and Kenefick, 2011, Gordon, 2017, Iba et al., 2022). Myofibrilar degradation can occur during LHS due to tissue catabolism stimulated by the need for glucogenic substrate, and as a result of hyper-inflammation as discussed in stage 3 below. There are no published case reports of LHS or associated observations of renal deterioration in mature cattle (Sullivan and Mader, 2018). Experimental induction of LHS in yearling Holstein steers did show degeneration of renal tubules, glomeruli mesangial cells, and urinary bladder and adrenal parenchyma congestion (Terui et al., 1980). Renal tubule degeneration has been reported in cases of LHS in other ruminants (young sheep) (Sula et al., 2012, Sprake et al., 2013). Renal dysfunction is commonly associated with heat stroke in dogs (Bruchim et al., 2017a) and in humans (Leon and Helwig, 2010).

LHS Pathogenesis Stage 3: Systemic Dysregulation & Degeneration:

Hyper-inflammation:

In addition to pro- and anti-inflammatory cytokines, pro-oxidants are generated due to hypoxia in ischemic tissues, thermal damage to tissues, and endotoxins. Systemic inflammatory response syndrome **(SIRS)** is a condition of systemic hyper-inflammation that occurs in LHS. It is characterized as a "cytokine storm", where the pro- and anti-inflammatory immune responses compete and create an imbalanced state that is often termed "out of control". In LHS, hyper-inflammation is caused in part by high systemic levels of endotoxin. Toll like receptors **(TLR)** are immune system proteins that recognize and bind pathogens. Endotoxin binds to toll like receptor 4 (**TLR4**) which stimulates many different cytokines, including tumor necrosis factor-α (**TNF**-α), interleukins (**IL**) IL-1 and IL-6 (Seeley et al., 2012). Many, many immune system modulators are involved in HS and endotoxin responses; both TLR4 and TNF-α exemplify this type of concurrent counteracting, causing as well as inhibiting immune responses. For instance, TNF-α, strongly pro-inflammatory, also induces anti-inflammatory activity such as IL-6 which can effect both pro-inflammatory and anti-inflammatory responses.

Oxidative Stress:

TNF-α primes phagocytes, including Kupfer cells to produce pro-oxidants, both reactive oxygen species (**ROS**) and reactive nitrogen species, (**RNS**). At low amounts these pro-oxidants have an important role in cellular signal transduction and redox regulation. In modest amounts these pro-oxidants are protective against cell damage by pathogens, but at prolonged elevated levels such as in endotoxemia they can trigger cell death (Halliwell and Gutteridge, 2015). In a short term HS study (24 hours), ongoing HS resulted in an increased systemic load of LPS (Wang et al., 2011) and net decrease (consumption) of antioxidants available in splanchnic tissues. Wang et al. cite similar HS associated reduction of anti-oxidant activity in other studies in goats, pigs, broilers, and dairy cows. However, Wang et al. note that some previous studies incorrectly measured increases in oxidative indicators, and therefore incorrectly concluded that oxidative stress increased during HS. Wang et al. attribute those errors (conclusions of increased oxidative stress) to measuring concentrations in blood, without considering simultaneous

decreases of blood flow, which could mean net increases may not have occurred in those studies. Wang et al. also conclude that in the trial they report net oxidative stress decreased when both concentration and flux are considered. Clearly, further assessment is needed of the effects of duration and severity of HS on oxidative stress, and of the measurement approaches used in Wang and other studies. Importantly, examining the appropriate level of dietary antioxidants supplied during severe HS in cattle should be a high priority.

Dysregulated Inflammation (SIRS):

As severe HS continues, increasing systemic endotoxin levels, ongoing hypoxia, and thermal tissue damage all facilitate progression of immune and inflammatory responses to pathological dysfunction levels that increase the risk of death. Systemic Inflammatory Response Syndrome (SIRS) is a hyper-inflammatory immune response that develops in cattle exposed to prolonged and severe hyperthermia. Similar to sepsis, SIRS differs in that it is defined with an expanded set of causes (Jaffer et al., 2010, Berg and Gerlach, 2018). Sepsis is the response to systemic infection caused by biological pathogens like microbes or viruses, whereas SIRS causes include the infectious causes of sepsis, but also non-biological causes of tissue damage such as trauma, burns, thermal damage, major surgery, or HS. The “out of control” (Jaffer et al., 2010) hyper-inflammatory response is a hallmark of SIRS. The severity and impact of dysregulation of the immune response is proportional to the extent to which pro- and anti- inflammatory cytokine counter-activation is systemic, as opposed to localized only to areas of cellular damage (Seeley et al., 2012). Dysregulation extent also depends on the magnitude of the cytokine responses because there can be thermal and hypoxic tissue damage generating immune responses in many tissues simultaneously.

Coagulopathy:

During severe HS progression to LHS involves development of dysregulated coagulopathy (clotting disorders)., Endotoxin stimulates endothelial (blood vessel wall) injury which activates hyper pro-coagulation elements causing thrombi that in turn activate anti-coagulation processes that inhibit thrombosis (clot) formation, causing bleeding. The effect is initially a compensated coagulation (coagulants offset the coagulation) followed by decompensated and unregulated hemostasis as anticoagulants are consumed. Then excessive coagulation occurs resulting in systemic disseminated intravascular coagulation **(DIC)** (Bruchim et al., 2017b). DIC is characterized by both systemic vascular thrombosis, and as clotting factors are consumed, vascular hemorrhage also. Very high heat alone (43^{o}C / 109^{o}F) activates some coagulation (Gader et al., 1990, Mohanty et al., 1997, Bruchim et al., 2017a). Both pro- and anti- coagulant factors, along with an important role of platelets (thrombocytes), contribute to vascular hyper-permeability. Endotoxins bind to a receptor complex,TLR4 and MD-2, (Ohto et al., 2012) which activates platelets and promote adhesion of platelets and neutrophils to endothelial cells (cells lining the walls of the blood vessels). Endotoxin also stimulates TLR in endothelial cells, enhancing coagulation, increasing platelet accretion, and resulting in the formation of microthrombi (small aggregates of platelets, fibrin, and red

blood cells, i.e., tiny clots). Microthrombi impair perfusion through small vasculature, including arterioles, capillaries, and venules. As the continuously increasing endotoxin load becomes overwhelming, endothelial cell activation causes endothelial cell death, which results in vascular permeability, which further intensifies both inflammation and coagulation. This ongoing and conflicted vascular response to endotoxemia contributes to intravascular coagulation (DIC) systemic organ dysfunction, and ultimately death.

Disseminated Intravascular Coagulation (DIC):

Opposing processes of pro- and anti- coagulation generate DIC, known historically as "consumptive coagulation". Systemic inflammatory responses stimulate cytokine production (Bruchim et al., 2008), especially the immune modulators interleukins (IL-1) and (IL-6) and tumor necrosis factor (TNF). Systemic DIC occurs predominantly in the microvasculature, and can result in organ tissue ischemia and organ dysfunction (Boral et al., 2017). Tissue factor (thromboplastin, an enzyme that converts prothrombin to thrombin) initiates DIC by causing excessive thrombin production (Stokol, 2012, Boral et al., 2017). Thrombin is a protease enzyme that converts soluble fibrinogen to fibrin, facilitating blood clot formation; an excess of thrombin results in microvascular occlusion in arterioles and capillaries. Acute DIC consumes platelets, resulting in a low platelet count (thrombocytopenia) and potentially increasing prothrombin time (**PT**) and activated thromboplastin (**aPTT**) times (longer PT and aPTT indicate extensive clotting). As the extent of excessive intravascular coagulation increases, activity of a main anti-coagulant factor, antithrombin (**AT**), is inhibited. However, as more fibrin clots are created, fibrinolysis is stimulated by the enzyme plasmin and begins to break down fibrin clots (Bruchim et al., 2008, Chapin and Hajjar, 2015). The net effect of platelet consumption and fibrinolysis is an increase in extravascular bleeding. Vascular permeability also may result in an efflux of fibrin into the extravascular space; this efflux may also be promoted by histamine (Burhans et al., 2022). Concurrent dysregulation of coagulation and fibrinolytic systems, i.e., hyper-coagulation (clotting) and hyper-fibrinolysis (bleeding), is a hallmark sign of DIC coagulopathy (Iba and Levy, 2020). Common clinical indicators of DIC are petechia and purpura (tiny and small hemorrhages) visible in mucous membranes and organs post mortem. Like processes during SIRS, the body's conflicting regulatory processes are competing, in this case pro- and anti- coagulation.

LHS Pathogenesis Stage 4: Lethality: Multiple Organ Dysfunction Syndrome, Acute Respiratory Distress Syndrome, Septic Shock

Multiple Organ Dysfunction Syndrome (MODS):

Complex, MODS is not uniformly or precisely defined in human medicine (Burhans et al., 2022), even less so in veterinary species (Osterbur et al., 2014), especially large animal production medicine. Precipitated by HS, consequences including thermal trauma, SIRS, DIC, and sepsis, result in MODS, as reported in many species, including humans, dogs, and laboratory animals. As LHS progresses through SIRS and DIC, multiple body systems become dysregulated and dysfunctional to the extent they are unable to maintain homeostasis without intervention (Nyström, 1998, Osterbur et al., 2014). Largely a

sequela to vascular endothelium activated to be pro-inflammatory, the primary cause of MODS is DIC hyper-coagulation in the microvasculature. However, organ tissue ischemia and thermal damage also contribute to MODS pathogenesis (Iba and Levy, 2020). Elevated levels of both pro- and anti-inflammatory cytokines are correlated with organ failure and death in animal and human heat stroke. Occurring in multiple organs, MODS especially affects the lungs, kidney, heart, liver, adrenals, and GIT (Bouchama et al., 2005, Osterbur et al., 2014, Boral et al., 2017).

Acute Respiratory Distress Syndrome (ARDS):

Acute Respiratory Distress Syndrome (ARDS) has been linked to heat stroke in humans (el-Kassimi et al., 1986, Bouchama et al., 1996, Tulapurkar et al., 2012) and in dogs (Bruchim et al., 2009). Like SIRS, DIC, and MODS, ARDS pathophysiology involves pro- and anti-inflammatory immune responses to inflammation which become unbalanced responses. Triggering events for ARDS can be SIRS or sepsis, presumably originating from the endotoxemia in LHS. Tissue damage from other causes as described above likely contribute. Similar to DIC coagulopathy, ARDS is precipitated by vascular injury (Matthay and Zemans, 2011) and activation of the pulmonary vascular endothelium resulting in derangement of coagulation. This generates hyper-coagulation in pulmonary and alveoli microvasculature (Dunkel, 2015). Activated platelets, neutrophils, and macrophages leak out from the microvasculature into extravascular tissue, and release pro-oxidants, proteases, and cytokines which intensify ongoing inflammation. This causes degeneration of both the alveolar endothelial and epithelial barriers, which induces pulmonary edema. Damage to the pulmonary interstitium and alveolar walls further impairs pulmonary function, causing hypoxia and hypercapnia (abnormally high blood CO_2). Severe damage to the alveolar epithelium is associated with respiratory failure and high mortality. Mortality risk from ARDS increases with the extent of MODS and other extant comorbidities (Matthay et al., 2012). Like SIRS or DIC, there is a paucity of research on ARDS pathogenesis in ruminant LHS. Neither pulmonary epithelial injury nor alveolar edema occurred in sheep dosed with endotoxin (Wiener-Kronish et al., 1991, Matthay and Zemans, 2011). However, Wiener-Kronish et al. used a 4 and a 24 hour exposure to endotoxin, but suggested that a more prolonged exposure (as during LHS) could possibly result in alveolar barrier function injury and edema. Whether the alveolar epithelium of bovines is resistant to degradation when exposed to LPS remains a research need.

Septic Shock (SS):

Septic shock (SS) is a subcategory of sepsis and SIRS distinguished by extreme circulatory, cellular, and metabolic abnormalities which intensify mortality risk (Singer et al., 2016); SS can be a terminal consequence of LHS. Septic shock is one of several types of cardiovascular shock, which has several different causes (Mosier, 2022). The form of SS in LHS is maldistributive shock (Constable et al., 2017b) associated with initial blood redistribution to the periphery stimulated by the need for cooling described earlier. Septic shock induced by uncontrolled endotoxemia generally has two phases, an initial hyper-dynamic phase of increased cardiac output, and a later hypo-dynamic phase of

reduced cardiac output (Constable et al., 2017a). Early on, cardiac output is increased by increased heart rate with a stroke volume similar to that prior to heat exposure, and by increased cardiac contractility (greater fraction of cardiac volume is ejected). The later deteriorating phase is characterized by systemic DIC, MODS, ARDS, reduced venous return volume, decreased cardiac contractility, decreased cardiac output, increased systemic arterial hypoxemia, and decreased arterial pressure. Loss of cardiac function leads to a moribund state. Septic shock in LHS is an outcome of systemic hyper-inflammation induced by endotoxin- stimulated TNF-α, IL-1 and other cytokines (Mosier, 2022) as described above in stage 3. These dynamic disorders in LHS do not occur in every case, nor in a consistent order. End stage SS can occur as a consequence of SIRS and ARDS, or of prolonged direct cardiac damage, as seen in human cases (Zahger et al., 1989, Marchand and Gin, 2022). These systemic disorders (i.e. SIRS, ARDS, and SS) are similar manifestations of a common underlying syndrome of diffuse, nonlocalized multi-organ dysfunction or failure (Armstrong et al., 2018) that ultimately is caused by the systemic and dysregulated hyper-immune response. In the end, the tissue hypoxia, “cytokine “storms”, and overwhelmingly dysregulated responses induce organ dysfunction and ensuing cellular and tissue injury in essentially all organ systems, then death results.

Suggested Epidemiologic, Clinical, and Diagnostic Information For Assessing LHS Probability In Cattle

LHS is a complex disorder in cattle veterinary medicine that currently has no working definition. Recognition that heat causes cattle deaths is only made when large numbers of cattle deaths are associated with a simultaneous severe heat event, as in the thousands of such deaths referenced here in the introduction. Death attributed to heat stress in cattle is made by association, without examining “How does heat cause cattle to die?” There are no published reports or studies that have investigated the specific signs, pathogenesis, clinical pathology, or post-mortem findings in mature cattle exposed to HS conditions that die. There are no cattle specific information on which to base valid, practical differential diagnostic approaches to LHS. Nonetheless, until research data is available, there is a need to review and suggest contexts, epidemiologic and observable signs, and diagnostics that can be useful. Such information will improve recognition of LHS as a potential differential diagnosis, and help assess the probability that LHS may or may not be occurring in individual animals, thus in a herd. No criteria or tests are definitive or pathognomonic (unique or decisive for a specific disorder) for diagnosis of LHS in cattle. Assessing probability of LHS requires compiling three types of information: 1) thorough history and context information, 2) clinical assessment and extent of signs in both affected and non-affected animals, and 3) triaging select situation-specific diagnostic tests that can be the most useful. Diagnostics and evidence of DIC coagulopathy can be useful when LHS is suspected. However, test panel results are not specific (Bruchim et al., 2008, Stokol, 2012), and vary with extent and progression of LHS at the time samples are obtained for testing.

Based on diagnosis of heat stroke in dogs and humans potential diagnostic options for LHS in a herd are listed below. Heat related injury (HRI) and DIC in animal species

other than bovine are well-studied disorders in veterinary medicine (Stokol, 2012, Bruchim et al., 2017a, Bruchim et al., 2017b, Hall et al., 2022). The published studies are useful in suggesting diagnostic information for HRI and predicting mortality in those species (Hall, E.J 2021). Although some diagnostic tests need to be validated and have reference intervals established for cattle; this could be accomplished in the near future if prioritized. Meanwhile, diagnostic decisions of LHS in cattle should be based on veterinary consultation, with a bovine veterinarian, a veterinary diagnostic laboratory, veterinary support specialists, and veterinary pathologists. Suggestions below are targeted at situation, context, and herd level assessment, which are essential to establish a probability that LHS is present. Suggestions listed for individual animal information are useful to support herd level information.

Suggested LHS Related Information: Epidemiologic, Clinical, and Diagnostic

History, Context, and Environment

1. History and context are essential and should include a detailed description of pertinent details and include a timeline highlighting the beginning and progression of the heat environment and cattle signs and behaviors: daily temperatures, humidity, THI (Zimbelman et al., 2009), night temperature (hot or cool?) wind speed if available. Do for A) 4 to 6 weeks prior to the heat event, and B) same as above for the 'runup' period (days marking the heat start) and for the duration of the heat event.
2. Micro environment of cattle location: Same data as above, actual or estimated
3. Micro environment of the cattle housing: daily-evening temperatures, ventilation, water access, cattle space/stocking density, quantify & rate these (Excellent, Adequate, Inadequate, Unsatisfactory).
4. Outside environment use: surface? duration? solar exposure? shade availability? water?
5. Animals bunching?

Affected Animals: Characteristics & Clinical Signs

1. Affected animals: Age, breed, DIM, haircoat color, location/pen, approximate # total affected in the group/pen, group/pen size.
2. Respiration Rate, panting score.
3. Physical examination, including demeanor, body temp, auscultation & heart rate
4. Recumbent / downer animals in established lactation?
5. Timeline of signs in animals: start, # affected over days/time.

Non-affected Animals: Characteristics & clinical signs?

1. Same as above on a set of clinically *non-affected animals* in same facility, but that may be representative of either less exposed or less affected baseline values.

Pen Observations: (10+ apparently affected cattle and suspect herdmates):
1. Prevalence of Respiration rate (_# elevated> 100? >120? >150?)?

2. Prevalence of _# open mouth panting? _# tongue extended? _# neck extended?
3. Percentage of fecal drops that are diarrhea (< 1 inch high, no defined margin).
4. Abnormal fecal characteristics: unusual color; blood present?: frank (red-hematochezia) or occult (black-melena).

Ante-mortem Diagnostic Options: Samples from clinical and clinical suspects:

1. Clinical chemistry (usually a standard bovine chemistry panel): liver & muscle enzymes, (gamma-glutamyl transferase (GGT), glutamate dehydrogenase (GLDH), sorbitol dehydrogenases (SDH), aspartate aminotransferase (AST), creatine kinase (CK)), albumin, blood urea nitrogen (BUN), creatinine, bilirubin. Alkaline phosphatase (ALP) (request if not on the standard large animal panel.)
2. Optional- separate cardiac troponin (cTnI).
3. CBC (Complete Blood Count) both whole blood in anticoagulant and dried blood smear slides for platelet count.
4. Coagulation Panel (interpretation requires the 1st 3 be run together): Fibrinogen, PT (prothrombin time), aPTT (activated partial thromboplastin time), D-dimer or FDP (fibrin degradation products), ATA (Antithrombin activity). Note: a single set of coagulation panels is likely inadequate; individual animals being monitored should be tested at initial veterinary evaluation and again 12 and 24 hours later. Results vary with extent, stage, and degree of damage/progression. Results are poor sensitivity early in heat stress injury and have good sensitivity as pathology progresses to severe HS. (Bruchim et al., 2017b).

Necropsy Gross Pathology Observations:

1. Key organs to assess: Rumen, GIT intestines and lumen contents, heart, lungs, kidney, liver, all mucous membranes, organ external and internal surfaces and tissue.
2. Organ circulatory pathology present?: vascular hyperemia; tissue congestion, swelling edema; hemorrhage, petechial, ecchymosis of organ external / internal surfaces; extravascular tissue edema; abdominal/thoracic/extravascular fluid or fibrin.
3. GIT epithelial and mucosal intraluminal lesions, i.e., ulcers, necrosis, mucosal hemorrhage, bloody fluid contents.

Necropsy Histopathology Tissue Submissions:

1. Key organs to assess: Heart, lungs, kidney, liver, intestine, muscle.
2. Organ and circulatory histopathology present?: micro-thromboses, fibrin, inflammatory cellular infiltration, necrosis, vascular dilation/engorgement, tissue congestion, tissue edema, muscle fiber degeneration, microscopic petechia, ecchymosis.

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