ASSESSING THE CONDITION OF ANIMALS DURING UNLOADING AT THE LIVESTOCK MARKET AND ITS EFFECT ON HEALTH AND WELFARE

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This is to certify that we have examined the above Master's thesis and have found that is complete and satisfactory in all respects, and that all revisions required by the thesis examination committee have been made

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List of abbreviations and symbols used

Abbreviations	Elaborations
CK	Creatine kinase
DLC	Differential leucocyte count
EDTA	Ethylenediaminetetraacetic acid
Ft	Feet
GDP	Gross domestic product
HAR	Human animal relationship
Hg	Hemoglobin
Km	Kilometer
LCM	Live Cattle Market
NEFA	Non-esterified fatty acid
PCV	Packed cell volume
Rpm	Rotations per minute
Sq ft	Square feet
TEC	Total erythrocyte count
TLC	Total leucocyte count
UV	Ultraviolet
Vs	Versus
°C	Degree Celsius
%	Percentage
>	Greater than
<	Less than
≤	Less than or equal to

Abstract

Domestic animals are transported for various reasons throughout the country, where loading and unloading are two of the most stressful events. Besides, livestock, vital to Bangladesh's economy, employs a significant share of the labor force. However, there is a scarcity of data regarding the effects of transportation stress on animals, particularly in goats. Additionally, there is a lack of comparative analysis across species in observed data related to transportation effects in Bangladesh. The current study observed animal behavior, physiological responses, and transport staffs' interactions during unloading of transported animal to identify transportation stress. A total of 8790 goats, 1207 cattle, and 712 buffalo belonging to 51, 75, 44 vehicles were monitored respectively, and data were collected through a structured questionnaire during unloading at the Sagorica Live Cattle Market in Chattogram. For each vehicle, the proportion of each parameter was calculated separately for clinical signs, animal behavior, and handlers' behavior. Clinical signs (7, 11, 11 parameters), animal behaviors (6, 6, 6 parameters), and handlers' behaviors (6, 5, 5 parameters) were assessed for goat, cattle, and buffalo, respectively. For each parameter based on the proportion, a vehicle was categorized into three such as, healthy, mild, and severe for clinical signs; mild offensive, moderately offensive, and frequently offensive for animal behavior or handlers' behaviors. For hemato-biochemical analysis, 40 blood samples were randomly collected from goats immediately after unloading at the Sagorica Live Cattle Market, and 20 samples were obtained from a well-managed goat farm serving as a control. The numbers of healthy parameters of clinical signs decreased with an increase of number of cattle ($P \leq 0.001$). The frequently offensive animal behaviors and frequently offensive handlers' behaviors were associated with an increase of number of animals in both cattle ($P \leq 0.001$) and buffalo ($P \leq 0.001$). However, the numbers of healthy parameters of clinical signs and mild offensive handlers' behavior increases with a decrease of stocking density in goats ($P \leq 0.001$). Cattle ($P < 0.05$) and goats ($P \leq 0.001$), loaded from the South West districts of Bangladesh, experienced potentially better handling practices or less stressful conditions. Meanwhile, cattle ($P < 0.05$) loaded from the North East districts of Bangladesh suggests areas for improvement in handling technique and animal welfare measures. There was a significant difference ($P = 0.00$) in all clinical sign variables within the species. Among clinical signs, old injuries (40.4±11.6), lacerations (23.4±7.3), and fresh bleeding (10.7±5.3) occurred at the highest frequency in buffalo, while nasal discharge was most common in goats (14.9±2.3) and cattle (8.9±5.3). Among animal behaviors, jumping occurred at the highest frequency (35.29%) in goats, while refusing to move was most common in cattle (20.30%) and buffalo (22.19%). There was a significant difference in all animal behavior variables (P=0.00) and in all handlers' behavioral variables (P=0.00) within the species. Among handlers' rude behavior, hanging by rope occurred at the highest frequency (38.89%) in goats, while beating was most common in cattle (23.45%) and buffalo (21.21%). Regarding hemato-biochemical parameters, significant differences (P=0.00) were observed in TEC, TLC, PCV, lymphocyte, monocyte, eosinophil, basophil (P=0.01), neutrophil-lymphocyte ratio (P=0.04), glucose, total protein (P=0.04), and creatine kinase (P=0.01). In conclusion, the physiological and hemato-biochemical changes observed in the study indicate a high level of transportation stress and substandard animal welfare, highlighting the importance of implementing and enforcing precise laws for animal transportation to mitigate stress and suffering.

Keywords: Animal welfare, Buffalo, Cattle, Goat, Hemato-biochemical, Transport, Handlers' behavior

Chapter 1: Introduction

Domestic animals are transported for reasons including sales, moving to slaughterhouses, herd replacement, participation in events like dairy melas and exhibitions, attending fairs and competitions, health checkups, treatment, and relocation for feeding and fodder (Ahmad Mir et al., 2019). Rural markets in Bangladesh act as the main sources of animals for trade, from which traders acquire and transport them to larger markets in various cities across the country (Hossain and Chanda, 2002). In Bangladesh, animals belonging to private traders are transported between markets and districts using trucks, walking on foot, boats, and occasionally trains (Alam et al., 2010a). Livestock is vital to Bangladesh's economy, employing 20% directly and 50% partially of the labor force, and contributing 1.85% to the total GDP and 16.52% to the agricultural GDP (Salim, 2023). Bangladesh experiences tropical weather with high temperatures (averaging between 30°C to 40°C) and humidity ranging from 60% to 85%, especially in the summer season (Alam et al., 2018). Animal transporters frequently have to move animals in harsh weather conditions, posing a threat to the animal's welfare due to the intense heat and high UV exposure experienced in Bangladesh's tropical climate (Alam et al., 2018). The well-being of an animal is influenced by its condition and how well it adapts to its environment (Broom, 2003a). Beausoleil and Mellor (2017) describe "Animal Welfare" as the subjective state experienced by an animal, which can fluctuate over time and span from extremely poor to exceptionally good. The term "animal welfare" is frequently used interchangeably with "animal wellbeing" and typically includes biological functioning (such as health, physiology, and productivity), affective state (the emotional and mental condition of the animal), and natural living (providing opportunities for the expression of natural behaviors) as outlined by Fraser et al. (1997), and Hemsworth and Coleman (2011).

The Human-Animal Relationships (HARs) is typically characterized as the extent of closeness or separation between humans and animals, encompassing their shared perception (Waiblinger et al., 2006). The nature of HARs plays a crucial role in shaping whether the impact on an animal's physiology and behavior is positive or otherwise (Zulkifli, 2013). Empathy holds a significant position in human connections and extends its influence to relationships between humans and other species, impacting the handling and care of animals (Colombo et al., 2017). Additionally, livestock handlers' attitudes towards animals are closely linked to their handling behavior, and harsh practices can adversely affect animal welfare, increasing animals' fear of humans (Ceballos et al., 2018). The behavior of animals like goats towards human handlers is influenced by the husbandry systems and early life experiences (Miranda-de la Lama and Mattiello, 2010). Le Neindre et al. (1996) observed that young animals lacking exposure to human handling exhibited increased fearfulness and occasional aggression towards farmers or handlers.

The transportation of live animals, a necessary practice in husbandry, is acknowledged as a significant cause of stress (Saeb et al., 2010). Stress, an environmental factor, disrupts an individual's control systems, leading to reduced fitness or well-being (Broom, 1986). Various stressors during transport include handling, loading, fasting, confinement, vibrations, changing light conditions, poor air quality, and introducing unfamiliar groups (Saeb et al., 2010; Zhong et al., 2011). Moreover, researchers like María et al. (2003) and Adzitey (2011) have identified factors such as duration, distance, loading density, weather, and road conditions as additional stressors in livestock transport. The stages of loading and unloading during transportation are inherently stressful, as indicated by research findings (Jacobson and Cook, 1998; Alam et al., 2010c). Notably, the act of loading induces more stress than unloading (Fisher et al., 2009), a contrast influenced by factors like the animal's rearing conditions, the level and nature of human-animal interaction, and the specific breed. In the study conducted by Fazio and Ferlazzo (2003), it was found that the well-being of animals during transportation is significantly influenced by stocking density. Research suggests that animals transported in densely stocked vehicles experience greater physiological stress reactions and compromised meat quality compared to those transported at moderate or low densities, as observed by Broom (2000). Animals can adapt to stress to some extent, but if the stress surpasses a certain point, they enter a state of distress (Bulitta, 2015). When an animal perceives stress from either an internal or external stimulus, it triggers physiological adjustments within the animal's body (Brown and Vosloo, 2017). Transportation stress induces notable physiological effects, including heightened adrenal cortical activity, compromised immunity, elevated morbidity, weight loss, and occasional mortality from infectious diseases (Maejima et al., 2005; Saeb et al., 2010).

The way animals respond to transportation stress involves intricate interactions between neurons and hormones, resulting in observable clinical effects (Minka and Ayo, 2013).

Animals display alterations in physical, biochemical, and immunological parameters (Buckham Sporer et al., 2007). The physical alterations encompass heightened body temperature, elevated heart and respiration rates, and more (Swanson and Morrow-Tesch, 2001). Biochemical modifications during transportation stress involve glucose levels, NEFA, muscle enzymes like CK, and others (Ishiwata et al., 2008; Uetake et al., 2009; Uetake et al., 2011). Indicators of dehydration, such as heightened PCV (Buckham Sporer et al., 2007) and serum protein levels (Buckham Sporer et al., 2008; Ishiwata et al., 2008), have been documented. The stimulation of the hypothalamic-pituitary-adrenal axis at different transportation stages results in noteworthy changes in cortisol and catecholamine levels, as proposed by researchers (Ishizaki et al., 2005; Buckham Sporer et al., 2007). When examining the immune response, transport stress leads to elevated counts of TLC and specific WBC types like neutrophils, eosinophils, and mononuclear cells in the bloodstream (Lomborg et al., 2007; Mitchell et al., 2008). Transportation decreases lymphocyte count and increases neutrophil count, elevating the neutrophil-to-lymphocyte ratio, thereby increasing vulnerability to diseases during transit (Mitchell et al., 2008; Hulbert et al., 2011). These alterations can act as effective biomarkers for estimating the extent of transportation stress (Fazio et al., 2012).

Transporting animals in regions with high temperatures and humidity, especially in tropical areas, presents more significant challenges compared to temperate regions, as noted by Minka and Ayo (2012). While most studies on goat transportation have focused on temperate regions (Rajion et al., 2001), efforts to mitigate transportation stress in goats during road journeys are still scarce (Galipalli et al., 2004; Minka and Ayo, 2007b). Furthermore, there is limited research on the behavior of transported animals and transport staff during unloading, comparative analysis of transportation-related data across species, and a shortage of hemato-biochemical data for transported goats in Bangladesh. So, the research was conducted with the subsequent objectives:

1. To investigate the behavior and physiology of transported goats, cattle, and buffalo during unloading.
2. To elucidate the behavior of transport staff during the unloading of animals.
3. To determine the correlation between the behaviors of goats, cattle, and buffalo, and staff behavior.
4. To assess the hemato-biochemical profile of transported goats as a stress indicator.

Chapter 2: Review of literature

2.1 Livestock market

Livestock markets are designated places equipped with specialized facilities, offering a space for buyers and sellers to gather for the purpose of trading live animals (Corrales-Hernández et al., 2018). Within the livestock market setting, various stimuli that induce fear in cattle are present, such as unfamiliar and startling elements, intense factors like loud noises, stimuli with evolutionary relevance like height above ground, isolation, darkness, alarming auditory and olfactory signals from fellow animals, along with instances of restricted access to food and water and the experience of unpleasant handling (Stojkov et al., 2020). Additionally, the nature of livestock market activities necessitates constant interaction between handlers and animals, and research indicates that animals may encounter distressing handling practices as part of their daily routines (Destrez et al., 2018).

2.2 Different means of transport

Livestock are transported via road, sea, or air for various purposes. In many countries worldwide, the predominant mode of transportation for all livestock species is by road (Brown et al., 1999a; Odore et al., 2004; Buckham Sporer et al., 2008). The vehicles used for cattle transportation vary widely, ranging from small trailers to specialized double-decker trucks, with rail being the least utilized means. Zulkifli et al. (2019) investigated the impact of sea and road transport on farm animals. Although air transport is less common than road transport, it remains integral to industry practices, particularly for animals involved in breeding. Walking remains a method of animal movement, prevalent in many developing countries, but it is notably stressful for the animals. Walking is suitable only in areas lacking roads or other infrastructure or for short distances between the farm and the destination. In Africa and Asia, substantial numbers of animals are walked for multiple days to reach markets or slaughter, frequently facing restricted access to food and water.

2.3 Fitness of animal for transport

The hazards during transport can pose significant risks, regardless of the health and physical fitness of the animals, depending on the journey's duration and quality. These risks become even more pronounced for animals that are weakened or vulnerable, as they may already be experiencing welfare issues prior to transportation. In such compromised conditions, these animals find it challenging to deal with transport-related hazards, including entering and exiting from vehicles, coping with other animals, ensuring stability, handling fatigue, addressing constraints on feed and water, and adapting to varying thermal conditions. To mitigate the potential for suffering during the journey, it is crucial to assess the animals' suitability for the intended trip before loading them. Animals should reach their destination in a state comparable to the assessment before loading, experiencing minimal decline during transit. Animals exhibiting conditions impacting their physical well-being and posing risks of exhaustion, weakness, or fatigue should not undergo transportation; instead, they ought to receive treatment or be euthanized at the farm. Taking into account the pathophysiological consequences of illness and injury on how an animal copes with the potential physical and physiological challenges during transport can help in recognizing the welfare concerns associated with transporting cattle unsuitable for the intended journey. Despite some cattle with health issues initially being deemed healthy at the start of a journey, there is a possibility of their condition deteriorating during transit (Dahl-Pedersen et al., 2018b). These animals are more prone to facing mortality during transport, becoming non-ambulatory, or requiring euthanasia upon arrival compared to their healthy counterparts (Cockram, 2019).

The criteria and conditions for declaring an animal 'unsuitable for transport' are likely to vary significantly among different types of cattle, presenting additional complexities for decision-makers, including farmers, livestock drivers, hauliers, veterinarians, and regulatory bodies (Dahl-Pedersen, 2022). This complexity not only poses challenges to the professionals involved but also raises concerns regarding the well-being of cattle that might undergo transportation despite being unsuitable for the planned journey. Evaluating suitability for transportation is a complicated task. Research in cattle has indicated uncertainty in the decision-making of professionals involved (Herskin et al., 2017; Dahl-Pedersen, 2022). A study comparing the suitability for transport of dairy cows among three professional groups (livestock drivers, veterinarians, and farmers) revealed, at most, moderate agreement (Dahl-Pedersen et al., 2018a).

The process of assessing suitability for transportation involves multiple elements and stages (Dahl-Pedersen, 2022). Initially, the herdsman/farmer/producer assesses and makes a decision regarding the fitness of the cattle. If there is uncertainty about the suitability, a clinical veterinary examination or consultation with a veterinarian may follow. The assessment of cattle's fitness for transport relies on either a checklist of general conditions affecting their ability to handle transportation or a list of specific conditions. If an animal is deemed unfit for transport, it should neither be loaded nor transported. Different stakeholders hold varied perspectives on the criteria used to determine the severity of conditions, such as lameness and poor body condition, that render an animal unsuitable for transport (Grandin, 2016). There exist potential conflicts in deciding whether to avoid the risk of suffering by choosing not to transport an unfit animal and the financial considerations tied to on-farm euthanasia. This contrasts with the potential benefits for a producer gained from transporting the animal for slaughter, enabling its utilization for human consumption. Balancing these factors presents dilemmas and involves weighing the ethical and economic aspects of the decision-making process. On-farm (emergency) slaughter options are limited, and there is a potential to transport the carcass to a slaughterhouse. When this alternative is accessible, it is likely to alleviate some of the challenges associated with decisions regarding suitability for transport versus resource wastage (Magalhães-Sant'Ana et al., 2017; Hultgren, 2018).

If an animal possesses a painful clinical condition before transportation, the act of transport is highly likely to intensify the pain, potentially leading to suffering. Movement or pressure on a painful area, such as an inflamed joint in arthritis, intensifies the pain. Therefore, actions like loading, unloading, responding to vehicular movements, interactions with other animals, and postural changes can induce movement in sensitive tissues, causing additional pain. Transporting animals with an unstable fracture is not advisable, as it can lead to heightened pain and potential suffering. Fractures in bones are inherently painful, with mechanical pressure on the fracture site or movement and distortion of the fractured bone causing discomfort (Cockram, 2019).

2.4 Assessment of animal welfare during transport

Over the past few decades, numerous scientific reviews such as Nielsen et al. (2011) and textbooks like Grandin (2019) have examined and deliberated on the implications of animal transport concerning animal welfare. By and large, there is a consensus that animal transport can result in significant adverse consequences for animal well-being. The evaluation of well-being during transport involves various metrics related to the animal's behavior (Cockram and Mitchell, 1999), metabolism (Hogan et al., 2007), stress physiology (Knowles and Warriss, 2000), and pathology (Chirase et al., 2004). Gathering information across a diverse range of measures is essential because transportation impacts various aspects of the animal's body and biological functions. Additionally, there are no precise threshold values that clearly distinguish normal stress responses from a compromised welfare state in animals (Moberg, 2000). During transportation, the primary physiological changes involve electrolyte imbalance, heightened respiration and heart rates, dehydration, energy deficit, and associated catabolism. Ideally, the journey conditions should be tailored to accommodate the breed, age, physiological state, and body condition of the animals, mitigating stress and its effects on their health and well-being (Nielsen et al., 2022). In the course of a lengthy journey, it is essential to provide animals with feed, water, and rest, adapting to their needs to prevent exposure to hazards that could result in welfare issues like prolonged hunger, extended thirst, and difficulties in resting. Extended periods of hunger may induce exhaustion and weakened conditions, while prolonged thirst can result in dehydration, discomfort, and suffering (Nielsen et al., 2022). Enhancing the understanding of how animals respond to diverse environmental conditions by animal producers, handlers, and transporters could contribute to improving the welfare of animals (Bulitta, 2015).

2.5 Road transportation stress in animals

The predominant stress types in today's livestock industry are technological stress and transportation stress (Broom, 2003a). While technological stress can be reduced, avoided, or entirely eliminated, transport stress remains an unfavorable and critical issue in the continually expanding global livestock industry. Road transportation is generally acknowledged as a stress-inducing factor for animals (Rajion et al., 2001; Giovagnoli et al., 2002; Buckham Sporer et al., 2008; Adenkola and Ayo, 2009;

Adenkola et al., 2009c). The severity of stress can vary based on factors such as crowding, temperature, deprivation of feed and water, and the duration of travel (Dalin et al., 1993; Ritter et al., 2007; Adenkola et al., 2009b). Road transport stress, or road sickness, refers to the stress condition animals acquire during road transportation, characterized by the violation of regulations governing standard transport welfare. This includes inadequate preparation before the journey, the impact of unfavorable climatic conditions, and management-related issues throughout transportation. The process of transportation disrupts the normal patterns of feeding and drinking in animals. The symptoms of road transport stress are generally consistent across various animal species (Parker et al., 2003). Nevertheless, highly productive, pregnant, and young animals tend to experience greater stress during road transportation compared to others (Broom, 2003a; Ferlazzo, 2003). The conditions of road transport are recognized to impact the physiological responses of animals, whether due to psychological stress or physical fatigue (Lambooy et al., 1985; Bradshaw et al., 1996). The susceptibility of animals to infection is influenced by transportation, as exposure to stressors during the journey is linked to a diminished immune system function. This impact is primarily facilitated by the hypothalamo-adreno-cortical system, involving the release of corticosteroid hormones that suppress the immune response (Warriss, 2004). The factors contributing to road transport stress are categorized into pre-transport factors (which encompass insufficient preparation before transportation), transport-related factors (including the length and duration of the joirney, climatic conditions, alterations in accustomed daily routines or stereotypes, road conditions, and vehicle speed), and post-transport factors (such as rough unloading of animals, substandard unloading ramps, insufficient food, water, and rest in lairage after transportation, and a deficiency of post-transport medication) (Hartung, 2003; Warriss, 2003; Minka and Ayo, 2007a, b).

2.6 Handling, loading and unloading of animals

The loading, transport, and unloading processes involved in transporting cattle place a substantial stress burden on the transported animals (María et al., 2004). The design of loading and unloading facilities is crucial. These operations are integral to the transport process and involve a series of practices for handling and guiding the animals onto or off the vehicle using various structures like fences, aisles, chutes, docks, ramps, and/or hydraulic lifts. The effectiveness of these operations is heavily influenced by factors within the production environment, including existing infrastructure, microclimatic conditions, the skills, training, and attitudes of handlers, as well as logistical planning (Pulido et al., 2019). Additionally, inherent factors related to the animals, including commercial category, past experiences, herding behavior of the breed, and suitability for transport, also impact the loading/unloading procedures (Miranda-de la Lama et al., 2014). The loading process may include practices that have the potential to induce stress, such as social separation, mixing, and physical restraint, all of which can affect cattle. The animals' response during unloading is contingent on factors like journey duration, road conditions, and microclimatic conditions (Lee et al., 2017). Delays in loading and unloading may occur due to various reasons, including facility issues, lack of organization, or untrained handlers. Any delay in loading/unloading during high temperatures and humidity increases the likelihood of heat stress. The alterations in body parameters of transported animals primarily occur due to handling, loading, and unloading (Minka and Ayo, 2009). Handlers and drivers of livestock vehicles should manage and load animals in a way that minimizes fear and enhances ease of handling. Practices like tail-twisting and the use of devices to compel animal movement (e.g., flags, electric prods, and sticks) can significantly influence the fear and stress levels of transported animals. The way these devices are applied, whether properly (with a soft touch, merely persuading), intense (stronger but not damaging), or rough (rude, with excessive force causing damage), plays a crucial role in the fear and stress experienced by the animals (Nielsen et al., 2022). Managing animals without the need for sticks or electric goads leads to improved well-being and reduces the risk of compromised carcass quality. A solid understanding of animal behavior and well-designed facilities are crucial for ensuring animal well-being during handling and loading. The identified welfare concerns during the loading and unloading of cattle encompass stress during handling, heat stress, injuries, and sensory overstimulation. In congested loading and unloading areas, ventilation is essential, and any unwarranted noise (such as motor sounds, yelling, or barking dogs) should be minimized or halted. Illumination during loading and unloading must be carefully planned to prevent stark contrasts between bright light and shadows. This includes strategic placement of lighting during nighttime operations to ensure uniform brightness over ramps, races, yards, inside the vehicle, and personnel access areas without glaring into the eyes of moving livestock. Additionally, in the morning and evening, it is advisable to avoid directing cattle towards intense light sources, be they artificial or from the sun (Nielsen et al., 2022). It is a frequent mistake to load animals early in the day, as this results in them being on the road during the hottest part of the day, given the often extensive distances covered (Petherick, 2005).

2.7 Effect of ramp during loading and unloading of animals

Insufficient availability of well-designed loading ramps poses a significant issue in various regions globally. This situation often leads to animals being tossed or compelled to leap from a vehicle elevated 1.2 meters above the ground (Bulitta et al., 2012). Loading and unloading infrastructure exhibit considerable heterogeneity in design and construction, influenced by the logistical innovation within the supply chain. Broadly, these structures fall into three categories: (1) fixed ramps or docks, (2) mobile ramps or docks, and (3) ramp systems and/or automated lifts (Nielsen et al., 2022). Calves, particularly those with limited pre-transport exercise, necessitate loading ramps with lower gradients. An optimal ramp angle of 20° is deemed suitable for cattle, provided there are surfaces with good traction and cleats positioned at 30 cm intervals (Nielsen et al., 2022). For cattle, pigs, and sheep, the recommended maximum angle for adjustable ramps is 25 degrees, while non-adjustable ramps should not exceed 20 degrees. Sheep exhibit ease in navigating steeper ramps in both ascending and descending directions. In the case of pigs, it is advisable to use ramps with an angle of 15 degrees or less (Berry et al., 2012). Animals' heart rate tends to increase with a higher angle of the ramp (Garcia and McGlone, 2014). In extensive stockyards, sales facilities, or slaughterhouses, having more than one loading or unloading ramp is typically necessary to expedite the process. The utilization of poorly designed handling methods and loading ramps contributes to an elevated risk of bruising (Garcia et al., 2019). Incorporating stair steps on concrete ramps is recommended, as they offer improved traction, especially when the ramp becomes worn or soiled. For loading cattle onto trucks, curved single-file ramps are particularly advantageous. A curved ramp featuring an inside radius of 5 meters is effective for both loading and unloading purposes. It is advised to avoid a shorter radius if the ramp is intended for unloading applications.

2.8 Impact of animal type and age during transportation

The suitability of animals for transportation and their ability to cope with the stress of the journey depend on the type of animal, encompassing factors such as age, condition, temperament, and previous experiences. Among these, age and weight play crucial roles in determining an animal's capacity to handle transport stress effectively. Moving un-weaned calves poses greater challenges compared to older cattle due to their lack of natural herding behavior, consequently increasing the risk of inadequate handling (Roadknight et al., 2021) and the potential for injury, discomfort, and pain during the handling process. Currently, a common practice involves transporting un-weaned dairy calves to a rearing facility at the age of 2–4 weeks. However, this timeframe aligns with a critical period in the calf's life concerning the maturation of its immune system (Hulbert and Moisá, 2016). The immune components of calves are not fully operational at 2 weeks of age, placing them in the so-called 'immune gap period' characterized by a decline in passive immunity and the lack of a mature adaptive immune system (Chase, 2022). Calves at a young age are documented to be more prone to stress during transportation compared to mature cattle (Eicher et al., 2006), leading to a greater occurrence of morbidity and mortality upon their entry into the feedlot (Fike and Spire, 2006). Supporting this, González et al. (2012b) discovered that calves were more prone to becoming non-ambulatory or facing mortality compared to fed and feeder cattle during transport. Younger calves haven't acquired the herding and following behavior seen in older cattle, making them more challenging to move (Jongman and Butler, 2013). Investigations also explore the body weight of calves as a factor in assessing transport risk, with results suggesting that calf weight significantly influences morbidity and mortality rates (Marcato et al., 2018). Masmeijer et al. (2019) observed that calves with lower body weight (< 46 kg; 2–4 months of age) exhibited leukocytosis and a higher prevalence of pro-inflammatory states after 2 hours of transport, as compared to their heavier counterparts (> 46 kg). Cernicchiaro et al. (2012) found that weight loss increased the susceptibility to bovine respiratory disease in lighter-weight calves in comparison to their heavier counterparts. Similarly, older animals, such as cull cows, face a heightened risk of compromised welfare during extended transport (>400 km) as they are more prone to becoming lame, non-ambulatory, and face mortality during and after the journey, in contrast to different groups of cattle (González et al., 2012b). Cernicchiaro et al. (2012) determined that heavier calves tend to recover more swiftly from transport due to their increased capacity to withstand stress compared to lighter-weight calves.

2.9 Influence of vehicle design during transportation

The primary factors influencing the welfare of farm animals during road transport include vehicle design, stocking density, ventilation, the standard of driving, and road quality (Fazio and Ferlazzo, 2003). The transportation of food animals using vehicles not expressly designed for this purpose can result in injuries, stress, and suffering for the animals (Jarvis and Cockram, 1994). Hence, it is advisable to avoid using vehicles such as open trailers, trucks, tippers, pickups, buses, and others with poorly designed ramps or doors that are excessively tight with unwanted projections. These vehicles, originally intended for transporting goods, are unsuitable for animal transportation. To mitigate the stress caused by the vehicle's design during sheep transport, Broom et al. (1996) recommended utilizing a 3.5-ton standard cattle lorry equipped with ventilation louvers. The selected mode of transportation should incorporate mechanical ventilation (regardless of engine type), and the internal temperature must remain above 5°C and below 30°C (Gavinelli and Simonin, 2003). Insufficient ventilation leads to heightened humidity levels and elevated concentrations of dust and harmful gases like NH_3, which are recognized as risk factors for respiratory disorders. Despite numerous modifications to transport vehicles and the recommended journey speed of 25-50 km/h, it remains evident that stress induced by the vehicle and its stocking density cannot be entirely eradicated (Minka and Ayo, 2009). This is primarily due to the fact that, while navigating turns and going uphill or downhill, animals not properly secured inside the vehicle often sway from one side to the other. Such animals may be pressed against the vehicle's sides, and in some cases, they may even fall, all of which compromises animal well-being. During the transportation of animals by vehicle, the vehicle's flooring should be slip-resistant, easy to clean and disinfect, well-drained, and devoid of urine or water to minimize injuries and the occurrence of fallen animals. Supplementing additional bedding on slippery floors can help mitigate resting problems during animal transportation (Nielsen et al., 2022).

2.10 Impact of driver behavior during transportation

The manner in which a vehicle is driven represents another crucial factor influencing animal well-being during transportation. The driver's proficiency significantly influences in the vehicle's movement and the subsequent balance of transported animals, especially during accelerations, braking, cornering, and other complex actions. Driver behavior comprises two key components: skill, which relates to the capability to control the vehicle, and style, which denotes the manner in which the vehicle is operated (West et al., 1993). European regulations mandate that drivers demonstrate skill and competence in animal transportation. The behavior of transported animals is influenced by both driver conduct and road conditions, whether on a highway, secondary road, or in urban traffic, as evidenced by studies involving sheep (Cockram et al., 2004), cattle, young calves, and pigs (Cockram and Spence, 2012). As a result, it has been proposed that factors such as vehicle condition (suspension, tire pressure), road quality, and the driver's expertise significantly influence the extent of stress related to transportation (Giovagnoli et al., 2002). Extended working hours, inadequate route planning, and disruptions to sleep patterns contribute to driver fatigue, increasing the risk of road accidents during the transportation of livestock (Miranda-de la Lama et al., 2011). Additional factors encompass the age of the driver, influenced by a mix of experience and overall well-being (González et al., 2012a). While animals have the ability to lie down and move in conditions of low stocking density during transport, their well-being may still be jeopardized if driving practices are suboptimal (Grandin and Gallo, 2007). Skillful driving can minimize issues for the animals, whereas poor driving quality can lead to welfare concerns, including difficulties in maintaining balance, motion sickness, and injuries (Broom, 2003a). Drivers are advised to employ gentle, defensive driving techniques, avoiding abrupt turns or stops, to reduce unpredictable movements of the animals. To achieve this, proper education for drivers is essential.

2.11 Outcome of stocking density during transportation

Stocking or loading density relates to the total weight of animals occupying a designated floor space (or sometimes the count of animals within a specific live weight range per unit area). The space allowance can be measured as the floor area allocated per animal. During transportation, animals necessitate a minimum space allowance that allows them to (a) accommodate their physical size, (b) modify their posture in response to acceleration and other events, (c) rest in a normal standing or lying posture, (d) thermoregulate, and (e) access food and water if provided in the means of transport. Loading animals in larger groups with limited space can heighten the risk of heat stress during transportation. The impacts of stocking density on animal well-being are among the most crucial considerations in the context of animal transportation (Hall and Bradshaw, 1998). For instance, when animals are unable to move and lie down comfortably due to elevated stocking density, it can negatively affect their well-being (Knowles et al., 1995). Increased stocking densities impede the proper balance of animals, elevating the risk of motion stress. The degree of stress encountered by sheep during transportation is impacted by stocking density (Cockram et al., 1996; Fisher et al., 2002; Fisher et al., 2010). Grandin and Gallo (2007) illustrated that elevated stocking density in cattle during transport diminishes carcass quality, leading to an increase in carcass bruising, severe injuries, or fatalities. High stocking rates during transport are linked to heightened muscle fatigue or damage in calves compared to lower stocking rates, as indicated by CK activity (Todd et al., 2000; Jongman and Butler, 2014). When stocking density is excessively high, animals lack the space to lie down and rise, escalating the risk of resting issues during journeys (Nielsen et al., 2022).

2.12 Influence of season, temperature, and humidity during transportation

The welfare risk for calves seems to be influenced by seasonal variations. The likelihood of seasonal mortality risk is highly contingent on the specific local climate. For example, winter has been identified as having the highest post-transport mortality rates in Canada and North Dakota (Winder et al., 2016), whereas late spring exhibited the highest transport mortality risk in New Zealand and Australia (Cave et al., 2005; Boulton et al., 2020). Extended transportation during the hot-dry season intensifies the occurrence of diseases in the transported livestock.

Extreme temperatures can lead to substandard welfare conditions for transported animals. The livestock industry has embraced the Temperature-Humidity Index (THI) as a weather monitoring tool to observe and mitigate production losses related to heat stress. In cattle, heat stress primarily results from elevated air temperature, exacerbated by factors such as high humidity (resulting from animals emitting water vapor), solar radiation (lack of shade), limited air movement, and metabolic heat. Solar radiation significantly contributes to heat stress and amplifies heat gain (Berman and Horovitz, 2012). Elevated ambient temperature combined with elevated air humidity can induce discomfort and elevate stress levels (Ganaie et al., 2013; Gonzalez-Rivas et al., 2020). To mitigate extreme temperatures, it is advisable to refrain from loading cattle during the peak heat hours of the day, especially if the vehicle lacks a mechanical ventilation system. Providing shade can shield animals from solar radiation and elevated ambient temperatures.

2.13 Effect of duration and distance of transport

Prolonging the duration of transportation can impact animal welfare by inducing fatigue, dehydration, and metabolic disorders due to stress during transit. The duration of a journey is typically more critical than the distance traveled (Warriss, 1990). Both the distance and duration of the journey are critical factors that impact animal well-being during transportation from the farm to the slaughterhouse (Gosálvez et al., 2006; Mazzone et al., 2010). The stress levels in sheep during transport are influenced by the duration of the journey (Fisher et al., 2010; Dalmau et al., 2013). Extended transportation distances have been found to result in a loss of live weight or carcass weight in animals (Brown et al., 1999a; Minka and Ayo, 2007b; Ritter et al., 2008). Goats, in particular, do not tolerate transportation stress well during long journeys. Reports from the industry, based on postmortem examinations, have suggested that goats become prone to respiratory infections, reduction in body weight, and compromised immune competency after extended journeys in unfavorable weather conditions (Kannan et al., 2000). Research indicates that the duration and distance of transportation, along with stocking density, impact the meat quality and welfare of various animals such as cattle (Broom, 2003b; Werner et al., 2013), lambs (De la Fuente et al., 2010; Ospina-Rojas et al., 2012; Sanchez-Sanchez et al., 2013), pigs (Gade and Christensen, 1998; Kim et al., 2004), and rabbits (Lambertini et al., 2006). Villarroel et al. (2003) and María et al. (2003) discovered that the duration of transportation impacts sensory aspects of beef meat, including tenderness and overall taste pleasantness. The transport distance was identified as a factor impacting transport-related mortality in dairy cows (Večerek et al., 2006) and fattened cattle (Malena et al., 2006) during transportation for slaughter in the Czech Republic. During the monitoring of long-haul cattle transport in North America, González et al. (2012b) observed that calves and cull cattle were more prone to mortality and becoming non-ambulatory during the journey.

2.14 Outcome of noise and vibration during transportation

Cattle, sheep, and pigs are capable of perceiving high-frequency sounds, and there's a possibility that they may be agitated or frightened by sounds that are beyond the range of human hearing (Fazio and Ferlazzo, 2003). Throughout the processes of loading, transportation, and unloading, various sounds perceptible to humans may occur from sources such as human voices, whips, animal vocalizations (including those of other species, e.g., barking dogs), noisy machinery, alarm bells/klaxons, and compressed air brakes on vehicles. While it's challenging for us to easily quantify all sources of ultrasounds, animals are subjected to numerous 'annoying' noises. Studies have shown that high levels of noise lead to excitation of the central nervous system, resulting in immune system suppression, fatigue, and cell death (Minka and Ayo, 2009). Excessive noise also increases defecation and aggression in animals, potentially leading to injuries.

Understanding vibration is a complex task, and investigating animal exposure comprehensively poses challenges. Vibration experienced on a load platform is influenced by factors such as road surface, speed, and suspension system. The unease induced by vibration during transport intensifies with prolonged exposure. The motion and vibration of the vehicle are recognized to impact the health, comfort, and postural stability of animals (Randall et al., 1995). Vehicle vibration has been linked to liver and muscle glycogen depletion, leading to fatigue in birds (Warriss et al., 1999); this effect may play a role in diminished animal performance or meat quality post-transportation. The body's reaction can be highly contingent on variations in frequency, making it essential to specify the frequency content of the vibration. Determining the vibration level is crucial for enhancing vehicle design (Bulitta, 2015). Vibrations possess significant potential to impact the welfare status of animals (Reynolds et al., 2019).

2.15 Impact of road transportation stress on blood physiology in livestock

2.15.1 Effects on hematological parameters

Elevated packed cell volume (PCV) can result from dehydration or splenic contraction triggered by sympathetic nerve activity or circulating catecholamines. During the handling and loading of animals, there is an observed rise in PCV values, while animals experiencing transport stress tend to exhibit decreased values (Tadich et al., 2005).

Varied journey times also show increases in hematocrit values, suggesting potential dehydration (Knowles, 1995; Tadich et al., 2005; Parker et al., 2007). However, Knowles et al. (1999a) reported a decrease in all transported calves, indicating a potential adaptation of cattle to handling over time (Tadich et al., 2005). It appears that stressors can genuinely lead to a decrease in hematocrit values, rather than just an apparent decrease. Adenkola et al. (2009a) illustrated that road transportation induces leukocytosis, neutrophilia, lymphocytosis, and eosinophilia in control pigs not supplemented with ascorbic acid.

Non-esterified fatty acid (NEFA) serves as a reliable indicator of the utilization of body fat, with its concentration rising in the bloodstream during periods of food deprivation in animals (Brown et al., 1999b; Kannan et al., 2002). There is an observed elevation in NEFA concentration corresponding to the duration of the journey in food-deprived animals. Additionally, Brown et al. (1999b) noted a significant two to threefold increase in NEFA concentration after 8 hours of transporting pigs, compared to the control group.

2.15.2 Influence on biochemical parameters

The stress factors encountered during transportation, particularly on hot days, lead to physical and psychological exertion, disrupting the homeostasis and, consequently, the metabolism in animals (Montané et al., 2002; López-Olvera et al., 2006; Averós et al., 2008). This exertion triggers an increase in the activity of hormones and enzymes, resulting in elevated blood levels of enzymes such as aspartate aminotransferase, alanine aminotransferase, glutamic phosphatase, and substances like glucose, creatine phosphate kinase, cortisol, nitrogen urea, lactic acid, uric acid, and free fatty acids (Apple et al., 1993; Parker et al., 2003; Parker et al., 2007; Ferguson and Warner, 2008). The activities of aspartate aminotransferase, alanine aminotransferase, and creatine phosphate kinase in the blood increase due to factors such as tissue damage, inadequate muscular tissue reperfusion, reduced heat dissipation, hypoxia, and fatigue. These changes seem to result from an elevation in muscle membrane permeability induced by the stress of capture, loading, and transportation (Knowles et al., 1999a; Montané et al., 2002; Tadich et al., 2005; López-Olvera et al., 2006; Guàrdia et al., 2009). Such increases are particularly evident when animals undergo rough handling and mistreatment before and after transport (Broom et al., 1996; Averós et al., 2008).

The stress induced by transportation is recognized for elevating plasma urea levels, indicating increased breakdown of proteins and nucleic acids in muscles. This occurs because of heightened cortisol concentration and prolonged food deprivation during stressful transportation conditions (Knowles et al., 1999b; Kannan et al., 2002; Guàrdia et al., 2009). Dehydration resulting from extended journeys has been linked to increased plasma total protein and albumin levels (Parker et al., 2003). A 19-hour road transport of calves showed a post-journey increase in total protein levels across all groups during both winter and summer periods (Knowles et al., 1999a). Plasma glucose serves as a common physiological indicator of stress during transportation (Knowles et al., 1999a; Broom, 2003a; Tadich et al., 2005; López-Olvera et al., 2006; Averós et al., 2008). Transportation-induced stress known to elevate plasma glucose concentrations through glycogen breakdown in the liver or depletion of glycogen stores in skeletal muscles (Kannan et al., 2000; Tadich et al., 2005; Averós et al., 2008). The elevation in plasma glucose concentration is primarily attributed to glycogenolysis associated with the surge in catecholamines and glucocorticoids secreted during transportation stress (Tadich et al., 2005).

Alterations in mineral metabolism induced by shifts in the original hormonal status due to environmental stress factors during animal transportation primarily affect calcium, magnesium, sodium, potassium, and chloride levels (Schaefer et al., 1997; Montané et al., 2002). Parker et al. (2003) noted the absence of noteworthy rises in calcium and phosphate ions in both control and experimental groups. Likewise, plasma concentrations of calcium, sodium, chloride, and phosphorous were noted to stay within normal values in transported cattle, steers, and calves (Atkinson, 1992; Parker et al., 2003).

2.16 Outcome of road transportation on behavioral activities of animals

The conduct of animals during transportation is a primary focus for both animal welfare enthusiasts and individuals involved in the livestock marketing industry. The behavioral responses of livestock during transportation are varied, largely contingent on perceived stimuli. The most significant stress typically arises in reaction to handling and loading procedures (Kannan et al., 2000; Kannan et al., 2002; Fazio and Ferlazzo, 2003; Ayo et al., 2006; Minka and Ayo, 2007a), particularly in animals raised under traditional extensive management systems. Behavioral alterations often serve as the initial indicators of disease and are key signs of distress. The captivity of animals, as occurs during transportation, has been noted to impose considerable stress, prominently reflected in behavioral changes (Minka and Ayo, 2007a, b). Animals undergoing behavioral stress during transportation express discomfort through various indicators such as freezing, backing off, attempting to escape, vocalizing, and kicking or struggling (Broom, 2003a). Behaviors like turning, mounting, fighting, changing position, and lying are predominantly observed in loose cattle, as tied animals have restricted movement (Bulitta, 2015). Loading in calves is often associated with slips and falls as the main challenges. Bravo et al. (2020) reported that slips and vocalizations were most common during unloading, while slips and turning around occurred most often during loading.

Chapter 3: Materials and methods

3.1 Location of the study area

The research was conducted at the Sagorica Live Cattle Market (LCM) in Chattogram, the second largest livestock market in Bangladesh (Alam et al., 2010a), located in Chattogram Metropolitan City Corporation at 22.3571° N and 91.7780° E, popular for trading goat, cattle, and buffalo throughout the year. Sagorica LCM is Chattogram's largest animal market, and the majority of animals are transported and unloaded there; hence, it was chosen as the study area.

Figure 3.1: Geographical location of Sagorica Live Cattle Market.

3.2 Study population

Goats, cattle, and buffalo that were transported from different parts of the country to Sagorica Live Cattle Market and unloaded were considered the study population. In 51 vehicles, 8790 goats were carried, irrespective of age, breed, and body weight. Among them, 7568 were male and 1222 were female. Irrespective of age, breed, and sex (mostly male), 1207 cattle and 712 buffalo were transported in 75 vehicles and 44 vehicles, respectively. Most of the animals were loaded from Pabna, Jessore, Sylhet, Kushtia, Chapainawabganj, Faridpur, Natore, and unloaded at Sagogica LCM.

3.3 Study duration

The research covered a duration of sixteen months, commencing in June 2022 and concluding in September 2023. The study mainly covered two Eid-Ul-Adha periods (July 2022 and June 2023), as more animals are transported during this time compared to other periods. Thus, most of the data were collected in June and July 2022 and June 2023. Additionally, data were also collected during the off-season of animal transportation since animals are transported throughout the year.

3.4 Data collection

A systematic survey sheet (Annex-I) was formulated related to the objectives of the study for the collection of data on transported animals at the time of unloading. The data included in the questionnaire were demographic information about animal loading, transport vehicle data, animal data, clinical signs and behaviors of animals during unloading, and the attitude and behavior of transport staff during unloading.

3.4.1 Observation of transport vehicles

An open-top truck is the most common mode of animal transportation in Bangladesh. There is no compliance with the design of the vehicle for transporting animals. Animals are directly exposed to natural adverse conditions, including direct sunlight in summer, cold in winter, and rain during the rainy season. The temperature and relative humidity were measured using a digital Thermo-Hygrometer (HTC-2, Tushmad, China).

3.4.2 Observation of clinical signs

Different clinical signs of transported goats, cattle, and buffalo were observed by close inspection of the animals during unloading.

Table 3.1: Definition of different clinical signs observed during unloading of animals

Clinical sign	Definition
Nasal discharge	It refers to abnormal fluid coming from animals' nose, often indicating underlying health issues such as infections, allergies, or respiratory problems.

Ocular discharge	It refers to abnormal fluid coming from animals' eyes, which can result from issues such as infections, allergies, trauma, blocked tear ducts, or foreign bodies.
Foamy mouth	It refers to the presence of frothy saliva or foam around the mouth of the animal.
Injury	
Old	An old injury in animals refers to a previously sustained trauma or damage to tissues, characterized by features such as scar tissue, changes in color, or altered function.
Fresh	A fresh injury in animals refers to a recent or newly acquired trauma or damage to tissues, specifically on the epidermal layer of the skin, characterized by inflammatory signs such as redness, swelling, heat, and pain.
Abrasion	Abrasion in animals is a superficial wound caused by friction, scraping, or rubbing of the skin surface, often involving the removal of the outermost layer of skin (epidermis), leading to mild bleeding, pain, and the absence of deep tissue involvement.
Laceration	A laceration in animals is a more severe type of injury characterized by a torn or jagged wound with irregular edges, involving deeper layers of skin and potentially extending into underlying tissues, such as muscles or tendons.
Fresh Bleeding	Fresh bleeding in animals refers to the recent release of red blood from a wound.
Lameness	Lameness in animals refers to a condition where an animal experiences difficulty or abnormality in its gait or ability to walk, often due to pain, injury, or musculoskeletal issues.
Abdomen	
Full	Full abdomen typically refers to an enlarged or distended belly.
Hollow	Hollow abdomen typically indicates a lean or undernourished state, characterized by insufficient body fat or muscle mass, resulting in a visibly concave or sunken appearance in the abdominal region.
Cleanliness of the body or skin	
Good	The presence of dirt on less than 20% of the body area.

Fair	The presence of dirt on 20-40% of the body area.
Poor	The presence of dirt on more than 40% of body area.

3.4.3 Observation of the behavior of animals during unloading

Different kinds of animal behavior were recorded during unloading after arrival at the animal market. These behaviors include refusing to move, jumping, falling, slipping, running away, and confusion.

Table 3.2: Definition of behaviors observed during unloading of animals

Observed behavior	Definition
Slipping*	Loss of balance in which the animal loses its foothold or the hooves slide on the floor surface. Only the hooves and/or legs make contact with the floor surface, with no involvement of other body parts.
Falling*	Loss of balance in which parts of the body other than feet and legs are in contact with floor surface.
Jump**	Leap with all four feet simultaneously off the ground in a manner or situation that could be hazardous.
Refuse to move/ reluctance to move*	An animal that refuses to move when coerced by the operator or that stops for at least 4 s not moving the body and the head (freezing).
Vocalization***	The animal makes a sound, emitted from the mouth, e.g. Bleating in goats and vocalization in lambs.
Grinding of teeth, curling of lips***	Teeth grinding and/or curling of upper lip.
Mount**	One animal stands on rear legs resting front legs and body on the back of another animal.
Aggression**	Forcefully contacting with another animal using the head or any other part of the body, like hitting or pushing.
Run away/turning around or moving backwards/turning back*	The animal turns around or moves backwards and run away from the handler (by itself or as a reaction to the handling), e.g. when arriving to the end of the unloading area or at the entrance to passageways.

Defecation/urination**	Elimination of faeces or urine from the body.
Confusion	Animal may stop and refuse to go forward, freeze, back away, run away, vocalize and /or show aggression at the same time.
(Nielsen et al., 2021)*; *(Bravo et al., 2020)*; ****(Goldberg, 2018)*.	

3.4.4 Observation of the attitude and behavior of transport staff during unloading

The handling practices of transport staff toward animals during unloading were observed, and their friendly and rude behavior was recorded in the questionnaire.

Table 3.3: Definition of different behaviors of transport staffs observed during unloading of animals

Observed behavior	**Definition**
Friendly	Friendly behavior in animals refers to positive, amicable actions, including expressions of affection, approachability, and non-aggressive social interactions.
Rude	
Beating	Beating in animals refers to aggressive actions involving repeated strikes or blows, often using objects such as wood, rope, plastic pipes, metal, etc.
Slapping	Slapping in animals involves striking with an open hand and can occur during aggressive encounters.
Pushing	Pushing of animals by handlers as a rude behavior refers to the forceful and disrespectful use of physical pressure to move or control an animal.
Hanging by rope	Tighten the animal's head with a rope and then bring it down from the vehicle to the ground by hanging it with the rope.
Dragging by ear	Dragging an animal by the ear refers to an inhumane practice of moving or pulling the animal by gripping its ear, potentially causing pain and distress.

Tail twisting and lifting	Tail twisting and lifting in animals by handlers are considered rude behaviors, involving the forceful rotation or manipulation of the tail, causing pain and distress to the animal.

3.5 Blood collection, transportation, and preservation

To determine the transport stress, blood samples were collected from transported animals. Blood was randomly collected from 40 goats immediately after unloading from the truck at the destination market (Sagorica LCM). Additionally, for analysis blood samples were collected from 20 healthy control goats reared on a farm under good management conditions. Some hemato-biochemical data related to transportation stress in our country are available for cattle and buffalo. However, no hemato-biochemical data of transported goats are available in our country, and due to limitations in funding, only goat blood was collected.

A blood sample of 6 ml was obtained from the jugular vein of each animal. The blood was then divided into two vacutainer tubes; one containing EDTA (anticoagulant) and the other without EDTA. The blood sample in the EDTA-containing vacutainer was used for hematological analysis, while the sample in the non-EDTA-containing vacutainer was utilized for biochemical analysis. The vacutainer containing EDTA holds 2 ml of blood, whereas the non-EDTA vacutainer holds 4 ml of blood. Then the vials were immediately placed in the icebox and transported to the laboratory of Chattogram Veterinary and Animal Sciences University within 1 hour of collection. Blood samples containing EDTA were stored at 4°C. Blood samples lacking anticoagulant were subjected to centrifugation at 3000 rpm for 15 minutes to isolate the serum from the blood. Then the isolated serum was placed in the eppendorf tube and stored at -18°C for subsequent biochemical analysis. All the laboratory tests were done within 24 hours.

3.6 Laboratory test

3.6.1 Hematological analysis

The blood sample in a vacutainer tube that contains EDTA was analyzed for total erythrocyte count (TEC), total leucocyte count (TLC), Hemoglobin (Hg), packed cell volume (PCV), and differential leucocyte count (DLC). Among them, DLC was done manually, and the rest of them were done by a fully automatic veterinary hematology analyzer (Celltac Alpha Vet MEK-6550 from Nihon Kohden, Japan).

3.6.2 Biochemical analysis

Serum glucose, total protein (TP), calcium, phosphorus, and creatine kinase (CK) levels were measured in serum samples utilizing a semi-automated analyzer (Humalyzer 3000, Germany) with the application of suitable kits commercially available.

3.7 Statistical analysis

3.7.1 Data management

All data gathered from the questionnaire and the results of the laboratory tests were recorded in Microsoft Excel 2013 (Microsoft Corporation, Washington, USA). After finalizing the data sheet, the data were imported into STATA 18 software (StataCorp, Texas, USA) for statistical analysis. Three separate datasets were prepared for goats, cattle, and buffalo, respectively.

3.7.2 Dependent and independent variables

Number of animals, platform, loading place, length of journey, and stocking density were independent variable whereas clinical signs, animal behavior, handlers' behavior and hemato-biochemical parameters were dependent variable. Among them platform and loading place were categorical variable whereas others were continuous variable. Nasal discharge, ocular discharge (for cattle and buffalo), foamy mouth (for cattle and buffalo), old and fresh injury, abrasion, laceration, fresh bleeding (for cattle and buffalo), lameness, full abdomen (for cattle and buffalo), and good cleanliness of the body were the parameters for assessing the clinical signs. Refuse to move, jump, fall, slip, run away, and confusion were the parameters for assessing the animal behavior. Friendly, beating, slapping, pushing, hanging by rope (for goats), dragging by ear (for goats), and tail twisting and lifting (for cattle and buffalo) were the parameters for assessing the handlers' behavior during transportation. For each vehicle the proportion of each parameter was calculated dividing number of animals with a parameter by total number of animals observed e.g., for nasal and ocular discharge portion was calculated dividing the numbers of animal with these signs by total numbers of animals observed in a vehicle. Based on the observation by the interviewer in each vehicle of transport the animals were assessed using the above-mentioned parameters to assess the dependent variables.

3.7.3 Definition of the dependent variables

Based on the variables of animal's clinical signs, animals were categorized into three types: a) healthy (if 0% of the proportion of the unloaded animal species had no unhealthy parameters) e.g., for clinical signs if the animals in a vehicle had 0% of a particular parameters, b) mild (if > 0% to 50% of the proportion of the unloaded animal species had unhealthy parameters), and c) severe (if > 50% of the proportion of the unloaded animal species had unhealthy parameters).

If the 50% value of the proportion and > 50% value of the proportion of the parameters remained the same and more than zero (0), then it was considered mild, and a higher value compared to that was considered severe. If the 50% value and > 50% value remained zero (0), then it was considered healthy, and a higher value compared to that was considered severe. Similarly, based on the variables of animal behaviors and handlers' behaviors, animals were also categorized into three types- mild offensive, moderately offensive, and frequently offensive, following the same process. But in some cases, like full abdomen, good cleanliness of the body, and friendly behavior of transport staff, 100% of the proportion of the criteria were considered healthy, < 100% to 50% were considered mild, and < 50% were considered severe.

3.7.4 Data visualization

Correlation tests were used separately for each animal species to analyze the relationship between clinical signs, animal behavior, and staff behavior concerning different journey-related variables. The data were visualized by determining the correlation for the clinical signs vs animal behavior, clinical signs vs handlers' behavior, and animal behavior vs handlers' behavior using the matplotlib and scipy.stats packages in Python 3.11.4 (Python Software Foundation, Wilmington, Delaware, USA).

3.7.5 Data analysis

Quantitative parameters of the animal's clinical signs, behaviors, and transport staff actions during unloading were compared using one-way ANOVA (analysis of variance), followed by multiple comparison using Bonferroni correction. For the comparison of quantitative hemato-biochemical parameters, a two-sample t-test (mean comparison test) for unequal variances was performed. The results were regarded as statistically significant at a threshold of $P<0.05$.

(a)

(b)

(c)

(d)

(e)

(f)

Figure 3.2: Different clinical signs of animal (a-d): (a) abrasion, (b) laceration, (c) fresh bleeding (d) old and fresh injury, (e) dead animal and (f) breathing inhibition in recumbent animal to make stand up.

(g)

(h)

(i)

Figure 3.3: Different behaviors of transported animals during unloading- (g) refuse to move, (h) jump, and (i) fall.

(j)

(k)

(l)

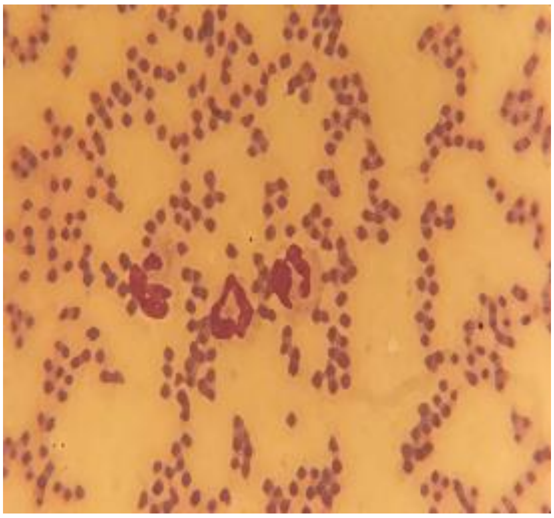
(m)

(n)

(o)

Figure 3.4: Different behaviors of transport staff during unloading of animals- (j,k) beating, (l) slapping, (m) pushing, (n) hanging by rope, and (o) tail twisting and lifting.

(p)



(q)

(r)

(s)

(t)

(u)

Figure 3.5: Hemato-biochemical analysis of the sample- (p) microscopic observation, (q) microscopic examination of blood film for DLC, (r) blood analysis by automatic veterinary hematology analyzer, (s) centrifugation of blood sample to collect serum, (t) serum sample, and (u) biochemical analysis.

Chapter 4: Results

4.1 Demographic and transportation related information

A total of 170 open trucks were observed for goats (51 vehicles), cattle (75 vehicles), and buffalo (44 vehicles). From the floor of the vehicle, the average length, width, and height of the truck were 19.38 ft, 7.95 ft, and 4.1 ft, respectively. In the case of goats, among the 51 vehicles during the study period, 35 came from Jessore, 11 came from Pabna, and 5 came from Meherpur. In the case of 75 cattle vehicles, 37 vehicles came from the South West area (Kushtia, Jessore, Jhenaidah, Chuadanga, and Rajbari), 22 vehicles came from the North West area (Chapainawabganj, Natore, Pabna, Sirajganj, and Rajshahi), 9 vehicles came from the South Central area (Faridpur and Shariatpur), 4 vehicles from the North East area (Sylhet and Kishoreganj), 2 vehicles from the North Central area (Tangail), and 1 vehicle from the Eastern Hill area (Chandanaish thana of Chattogram) of Bangladesh. In the case of 44 buffalo vehicles, 27 vehicles came from Sylhet, 7 vehicles came from Chapainawabganj, 5 vehicles came from Jessore, 4 vehicles came from Chuadanga, and 1 vehicle came from Kushtia. The range of the length of the journey was 400–480 km in the case of goats, 43–568 km in the case of cattle, and 355–568 km in the case of buffalo. According to the distance of the journey and the traffic conditions on the road, the duration of the journey varied from 12–17 hours for goats, 3.75–20.67 hours for cattle, and 11–18.5 hours for buffalo. The stocking density varied from 1.21–2.57 sq ft per animal in goats, 6.7–77.04 sq ft per animal in cattle, and 8.56–14.01 sq ft per animal in buffalo. The temperature ranged from 23.5–32°C in goat, 30.4–36.5°C in cattle, and 20–33.2°C in buffalo during unloading time, with relative humidity ranging from 43–77% in goat, 49–73% in cattle, and 54–73% in buffalo.

During the journey, the animals' head directions were varied, with some facing forward, some backward relative to the travel direction, and others perpendicular to the vehicle. No provision of feed or water occurred during the journey, and an open-top truck was used for animal transportation without any breaks being provided. Among goats, 15 (0.17%) were found dead.

Table 4.1 shows that the average number of goats, cattle, and buffalo in the vehicles was 190, 16, and 17, respectively.

Table 4.1: Frequency of the observations on numbers of vehicles and summary of animals per vehicle

Species	Number of vehicles	Mean	Median	Min-Max
Goat	51	172.35	190	60-230
Cattle	75	16.09	16	2-23
Buffalo	44	16.18	17	11-18

Mean and median were calculated for the number of animals per vehicle.

Table 4.2 shows that, in case of cattle and buffalo, 108 cattle (8.95%) and 106 buffalo (14.89%) were recumbent out of 1207 cattle and 712 buffalo. To help the recumbent animals stand up, the staff most frequently used tail lifting/dragging in cattle (60.19%) and beating in buffalo (65.09%). Conversely, the least frequently observed staff reactions were breathing inhibition in cattle (8.33%) and tail biting in buffalo (7.55%). Notably, in the case of recumbent buffalo, no instances of breathing inhibition were observed.

Table 4.2: Practices employed by the handlers to assist the recumbent cattle and buffalo in standing up

Variable	Recumbent Cattle (N=108)	Recumbent Buffalo (N=106)
	Frequency (%)	Frequency (%)
Shouting	30 (27.78)	20 (18.87)
Beating by wood/rope/plastic pipe/metal	58 (53.70)	69 (65.09)
Slapping	27 (25.00)	37 (34.91)
Lifting of or dragging by tail	65 (60.19)	54 (50.94)
Tail biting	19 (17.59)	8 (7.55)
Tail twisting	15 (13.89)	29 (27.36)
Breathing inhibition	9 (8.33)	-

4.2 Correlation between clinical signs, animal behavior, and handlers' behavior

Table 4.3: Univariable linear regression analysis of the association between the healthy parameters of clinical signs, mild offensive parameters of animal behavior, and handlers' behavior in cattle (N=75 vehicles) and buffalo (N=44 vehicles) with different journey related variables

Variable	Cattle			Buffalo		
	β^1	β^2	β^3	β^1	β^2	β^3
No. of animal	- 0.2**	- 0.2**	-0.03**	- 0.2	- 0.2**	- 0.03*
Platform						
Level	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Downward	- 0.3	- 0.2	0.01	- 0.8	- 0.02	- 0.07
Upward	- 0.2	0.97	0.3*	- 0.3	- 0.5	- 0.07
Loading place						
North West	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
South West	0.5	0.7*	0.1	- 0.1	0.1	7.5
North East	- 2.7*	- 0.3	- 0.05	0.1	0.06	0.04
North Central	2.04	0.5	- 0.05	-	-	-
South Central	0.2	0.4	- 0.05	-	-	-
Eastern Hill	3.5	1	- 0.05	-	-	-
Length of journey	- 0.002	- 0.002	0.0001	- 0.001	- 0.0004	- 0.0002

*[1] Correlation co-efficient (**β**) of the number of healthy parameters of clinical signs; [2] Correlation co-efficient (**β**) of the number of mild offensive parameters of animal behavior; [3] Correlation co-efficient (**β**) of the number of mild offensive parameters of handlers' behavior; * $P<0.05$; ** $P\leq 0.001$.*

Table 4.3 illustrates a significant negative correlation between the number of animals and the healthy parameters of clinical signs, mild offensive parameters of animal behavior, and mild offensive parameters of handlers' behavior in cattle and buffalo, except for clinical signs in buffalo, where the correlation was not significant. In the case of the platform, the level platform was considered the reference, showing a significant positive correlation between the upward platform and handlers' behavior in cattle. Regarding the loading place of animals, it was classified based on the hydrological regions in Bangladesh (Mojid et al., 2019), with the North West area considered as the reference. This classification revealed a significant positive correlation with animal behavior in the South West loading area and a significant negative correlation with clinical signs in the North East loading area in cattle.

Table 4.4: Univariable linear regression analysis of the association between the severe parameters of clinical signs, frequently offensive parameters of animal behavior, and handlers' behavior in goats (N=51 vehicles) with different journey related variables

Variable	Goat		
	β^1	**β^2**	**β^3**
Loading place			
North West	Ref.	Ref.	Ref.
South West	- 3.4**	0.04	- 4.08**
Length of journey	- 0.03*	0.004	-0.04**
Stocking density	5.4**	0.4	4.96**

*[1] Correlation co-efficient (β) of the number of severe parameters of clinical signs; [2] Correlation co-efficient (β) of the number of frequently offensive parameters of animal behavior; [3] Correlation co-efficient (β) of the number of frequently offensive parameters of handlers' behavior; * $P<0.05$; ** $P\leq0.001$.*

In the case of goats, no healthy and mild offensive parameters were found for clinical signs and animal behavior, respectively. Among the 51 vehicles, mild offensive parameters for handlers' behavior were found in 12 vehicles. Therefore, severe or frequently offensive parameters were used to determine correlation in goats.

Table 4.4 shows that there was a significant negative correlation between the length of journey and the severe parameters of clinical signs, and the frequently offensive parameters of handlers' behavior. There was a significant positive correlation between the stocking density and the severe parameters of clinical signs, and frequently offensive parameters of handlers' behavior. In the case of the loading place of animals, it was classified based on the hydrological regions in Bangladesh (Mojid et al., 2019), with the North West loading area considered as the reference. This classification revealed a significant negative correlation between the South West area and severe parameters of clinical signs, and frequently offensive parameters of handlers' behavior.

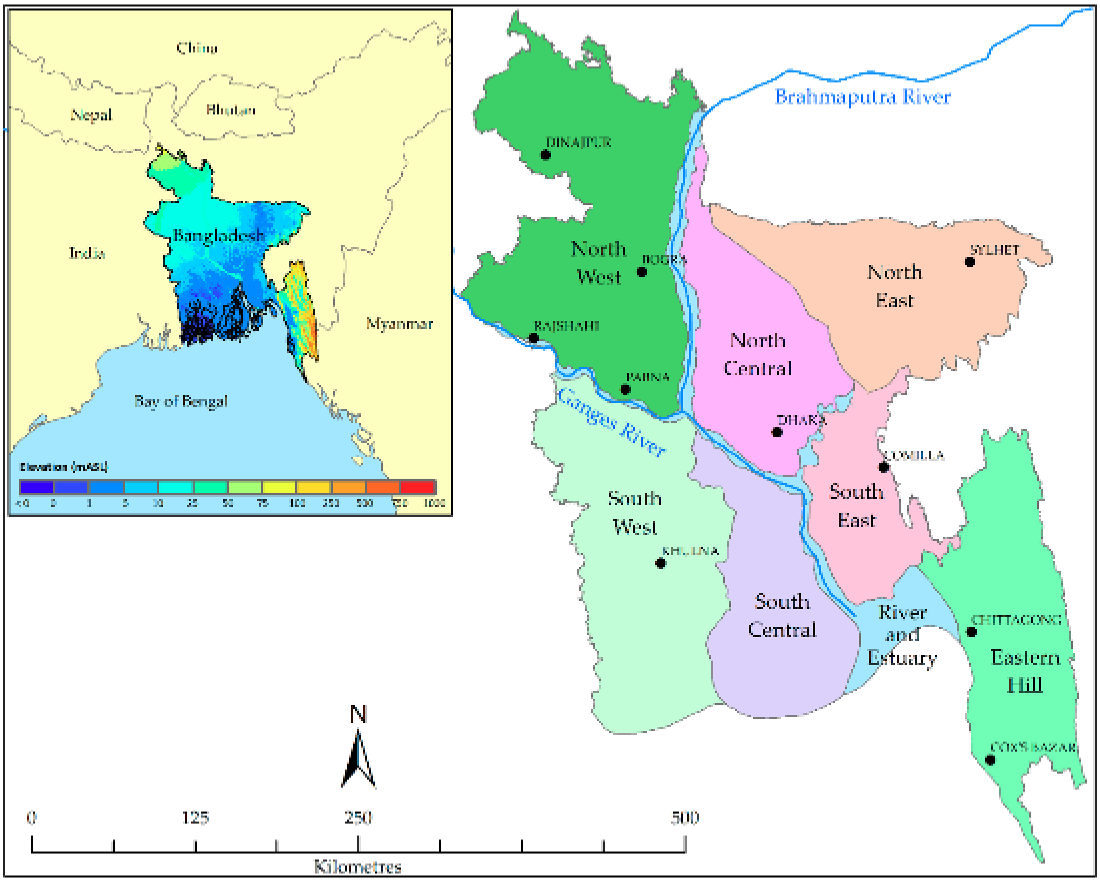


Figure 4.1: Hydrological regions in Bangladesh.

Figure 4.2 illustrates a significant positive correlation between clinical signs and animal behavior, as well as between animal behavior and handlers' behavior in cattle. Additionally, there was a positive correlation between clinical signs and handlers' behavior in cattle, although it was not significant.

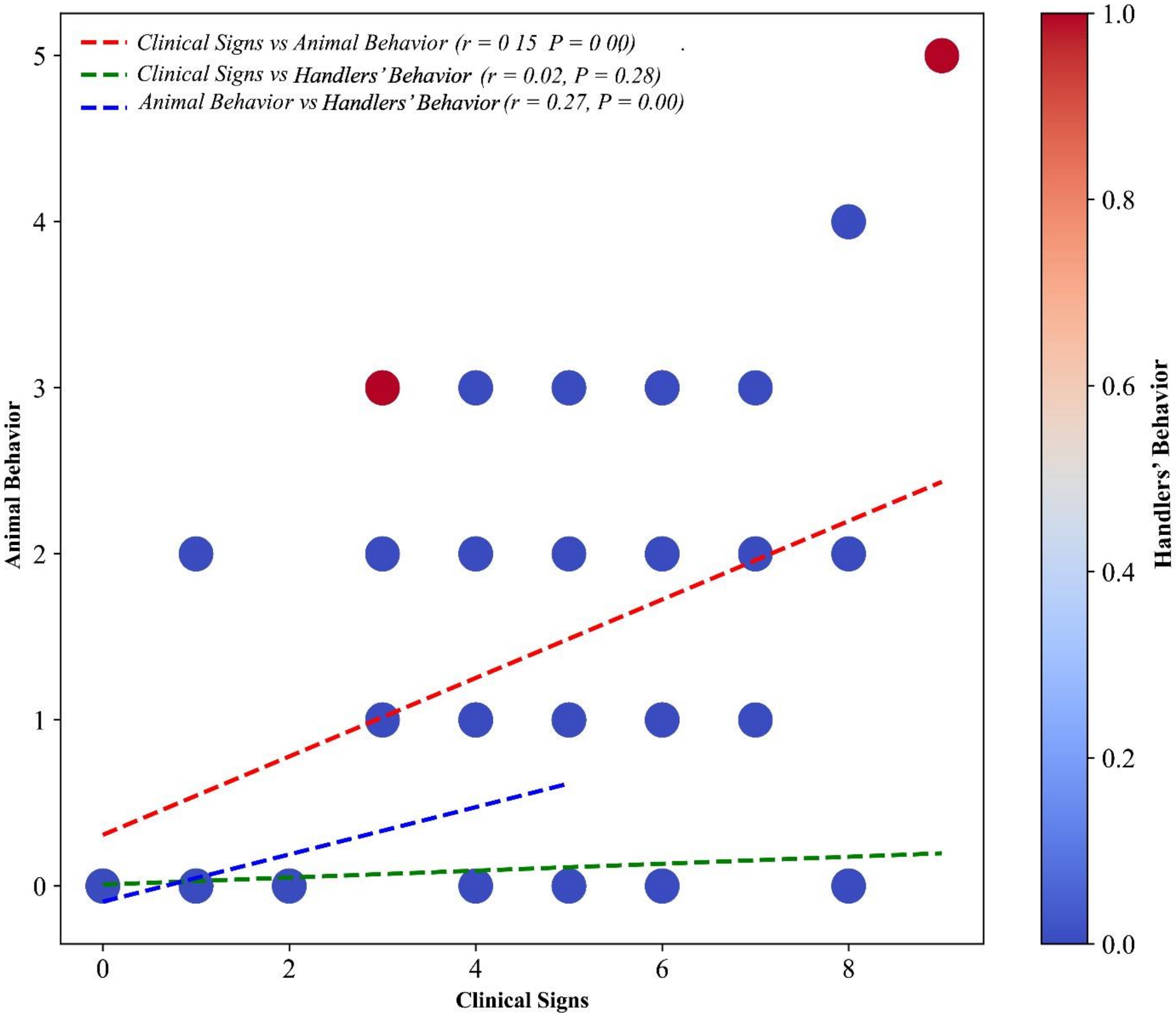


Figure 4.2: 2D heatmap of clinical signs, animal behavior, and handlers' behavior in case of cattle with correlation lines and p-values.

Figure 4.3 shows that in buffalo, a significant positive correlation was observed between clinical signs and handlers' behavior, as well as between animal behavior and handlers' behavior. Additionally, the correlation between clinical signs and animal behavior was positive, although not significant.

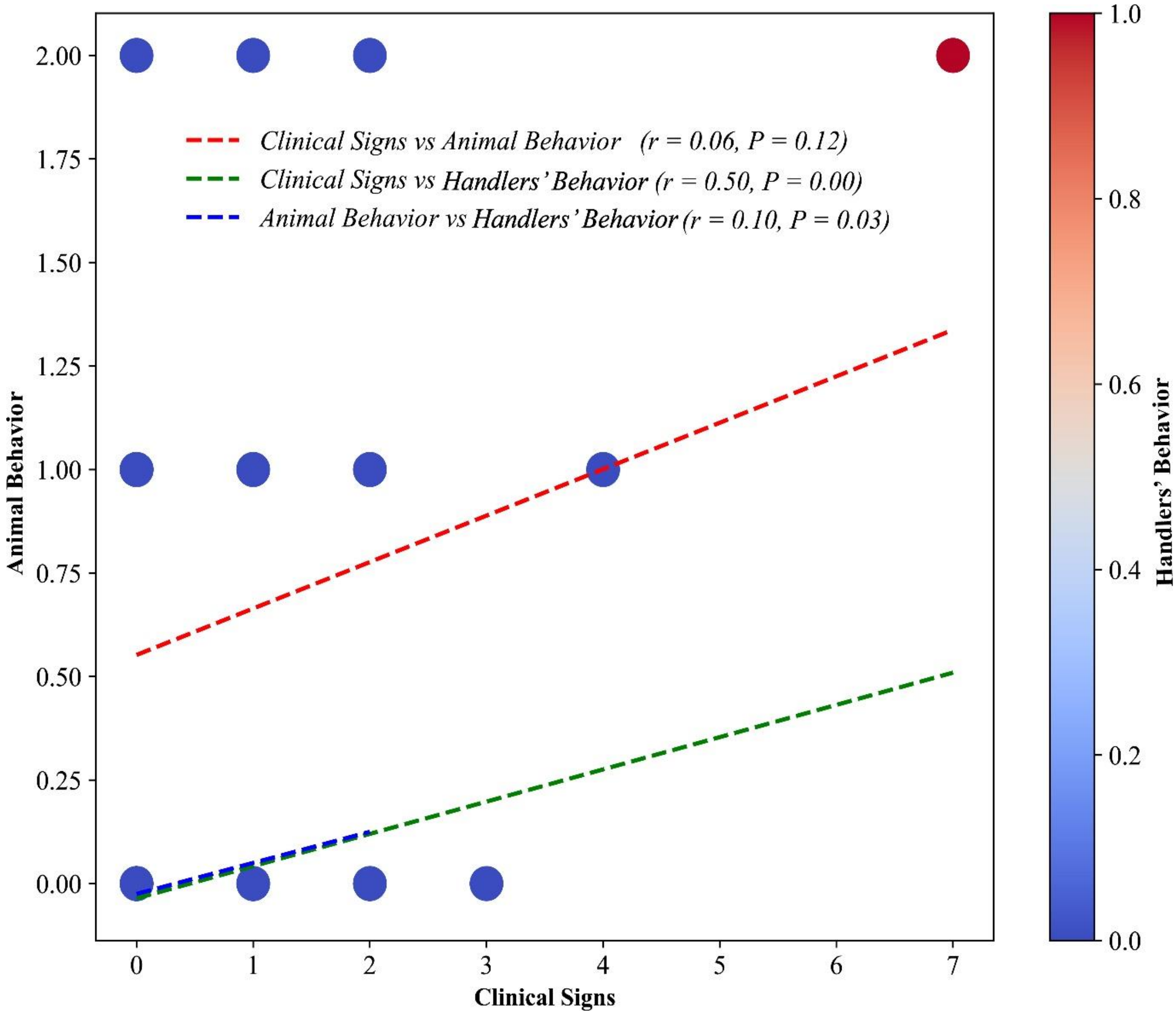


Figure 4.3: 2D heatmap of clinical signs, animal behavior, and handlers' behavior in case of buffalo with correlation lines and p-values.

4.3 Differences between clinical signs, animal behavior, and handlers' behavior with their significance level

Table 4.5: Differences in the clinical signs of the transported animals

Variable	Goat (N=8790)		Cattle (N=1207)		Buffalo (N=712)		P value
	PA (n)	Mean±sd	PA (n)	Mean±sd	PA (n)	Mean±sd	
Nasal discharge	1301	14.9±2.3[a]	103	8.9±5.3[b]	81	11.4±5.9[c]	0.00
Ocular discharge	-	-	56	4.5±4.1	61	8.6±5.7	0.00
Foamy mouth	-	-	74	6.3±5.1	90	12.8±6.9	0.00
Injury							
Old	473	5.9±2.7[ab]	41	3.2±4.7[ab]	290	40.4±11.6[c]	0.00
Fresh	1294	15.0±1.9[a]	64	6.1±7.5[b]	204	28.3±9.8[c]	0.00
Abrasion	821	9.7±1.5[a]	72	6.9±7.0[b]	134	19.1±5.1[c]	0.00
Laceration	549	6.3±1.5[a]	26	1.9±3.5[b]	167	23.4±7.3[c]	0.00
Fresh bleeding	-	-	16	1.1±2.5	76	10.7±5.3	0.00
Lameness	451	5.2±0.9[ab]	89	7.3±5.8[ab]	74	10.2±5.3[c]	0.00
Abdomen							
Full	-	-	951	81.6±18.7	467	66.1±10.2	0.00
Hollow	-	-	256	18.5±18.8	245	33.9±10.2	0.00
Cleanliness of body							
Good	3967	44.6±5.0[ac]	915	78.0±18.8[b]	337	47.7±12.3[ac]	0.00
Fair	2549	29.2±2.5[a]	66	5.3±11.0[bc]	57	7.8±9.5[bc]	0.00
Poor	2274	26.3±4.3[a]	226	16.7±14.9[b]	318	44.5±10.7[c]	0.00

PA = Number of Positive Animal; [a, b, c] Means with various superscripts within the same row differ significantly (P<0.05).

Table 4.5 illustrates a significant difference in all clinical sign variables within the species. In buffalo, the incidence of old injuries (40.4±11.6) and lacerations (23.4±7.3) was much higher than in other species. Fresh bleeding (10.7±5.3) was also more prevalent in buffalo compared to cattle.

Table 4.6 shows a significant difference in all animal behavior variables within the species. In goats, the incidence of jumping (35.6±1.7) and falling (18.4±1.5) was much higher than in other species.

Table 4.6: Behavioral differences of transported animals

Variable	**Goat (N=8790)**		**Cattle (N=1207)**		**Buffalo (N=712)**		**P value**
	PA (n)	**Mean±sd**	**PA (n)**	**Mean±sd**	**PA (n)**	**Mean±sd**	
Refuse to move	1635	$18.3±1.7^{a}$	245	$20.9±7.7^{bc}$	158	$22.3±4.3^{bc}$	0.00
Jump	3102	$35.6±1.7^{a}$	211	$17.8±12.1^{bc}$	149	$21.1±6.5^{bc}$	0.00
Fall	1637	$18.4±1.5^{a}$	82	$6.5±5.0^{bc}$	50	$7.0±4.4^{bc}$	0.00
Slip	1257	$14.0±1.6^{ac}$	106	$8.3±6.9^{b}$	91	$13.0±6.1^{ac}$	0.00
Run away	664	$8.0±2.3^{ac}$	63	$5.0±3.9^{b}$	59	$8.3±4.8^{ac}$	0.00
Confusion	480	$5.7±0.9^{ac}$	41	$3.2±3.4^{b}$	34	$4.8±3.8^{ac}$	0.00

PA = Number of Positive Animal; $^{a, b, c}$ Means with various superscripts within the same row differ significantly ($P<0.05$).

Table 4.7 demonstrates significant differences in all transport staff's behavioral variables within the species. Cattle exhibited notably higher levels of friendly behavior (40.5±8.6) compared to other species, whereas buffalo showed a higher incidence of tail twisting and lifting (17.6±5.6) compared to cattle.

Table 4.7: Behavioral differences of transport staffs during unloading of animals

Variable	Goat (N=8790)		Cattle (N=1207)		Buffalo (N=712)		P value
	PA (n)	Mean±sd	PA (n)	Mean±sd	PA (n)	Mean±sd	
Friendly	1349	16.3±3.4[a]	491	40.5±8.6[b]	229	32.2±6.0[c]	0.00
Rude							
Beating	1203	14.5±3.1[a]	283	23.6±7.7[bc]	151	21.2±5.1[bc]	0.00
Slapping	976	11.9±3.1[a]	200	17.0±6.3[bc]	132	18.7±5.6[bc]	0.00
Pushing	1367	16.6±3.8[ac]	163	14.1±6.5[b]	121	17.1±5.1[ac]	0.01
Tail twisting and lifting	-	-	118	9.7±5.0	124	17.6±5.6	0.00

PA = Number of Positive Animal; [a, b, c] Means with various superscripts within the same row differ significantly (P<0.05).

4.4 Frequency of animal behavior and handlers' behavior during unloading of animals

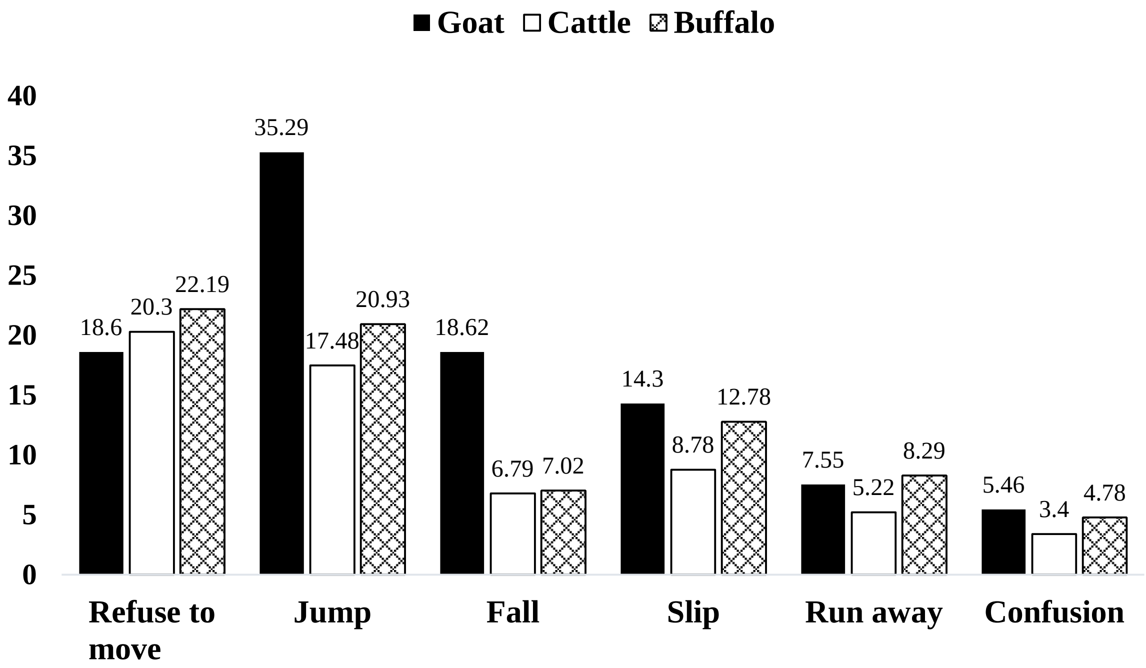


Figure 4.4: Frequency (%) of behavior of transported goats (N=8790), cattle (N=1207) and buffalo (N=712) during unloading.

Figure 4.4 shows that among animal behaviors, jumping occurred at the highest frequency (35.29%) in goats, while refusing to move was most common in cattle (20.30%) and buffalo (22.19%). Confusion was least frequent in goats (5.46%), cattle (3.40%), and buffalo (4.78%).

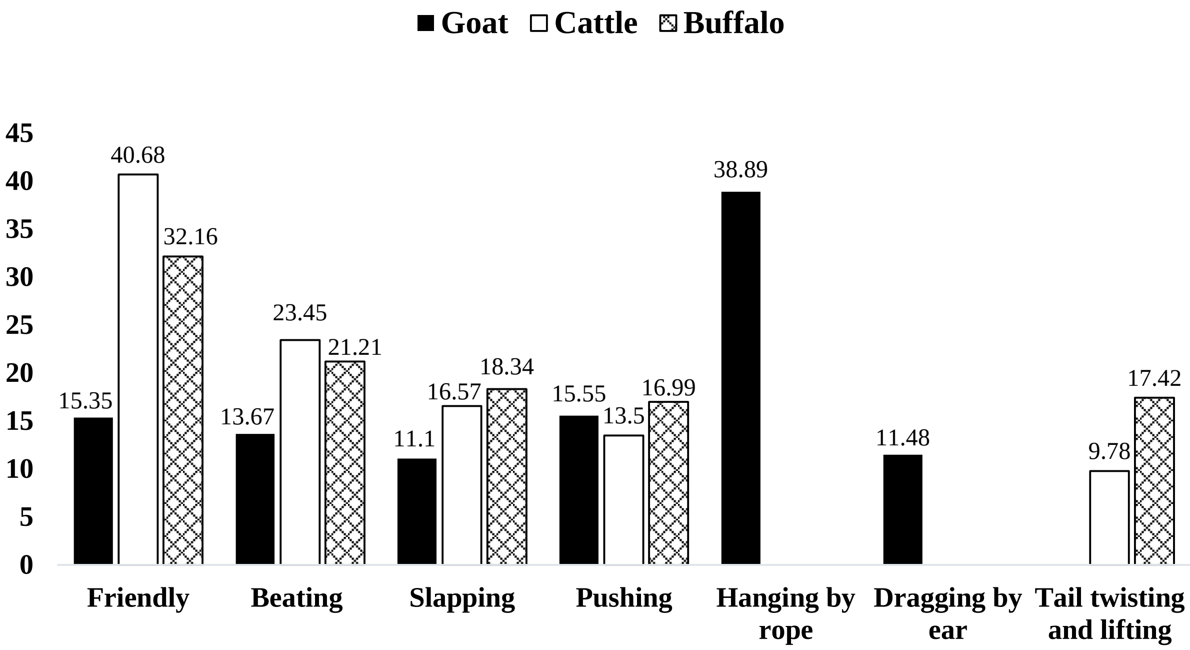


Figure 4.5: Frequency (%) of behavior of transport staff during unloading of transported goats (N=8790), cattle (N=1207) and buffalo (N=712).

Figure 4.5 shows that among transport staff's rude behavior, hanging by rope occurred at the highest frequency (38.89%) in goats, while beating was most common in cattle (23.45%) and buffalo (21.21%). Friendly behavior was observed in 15.35% of goats, 40.68% of cattle, and 32.16% of buffalo.

4.5 Hemato-biochemical information of transported goats

Table 4.8 illustrates a significant increase in TLC, monocyte, eosinophil, basophil, and neutrophil lymphocyte ratio (N:L) in transported goats compared to non-transported goats, except for Hb and neutrophil, where the difference was not significant. There was also a significant decrease in TEC, PCV, and lymphocyte in transported goats compared to non-transported goats.

Table 4.8: Comparison of mean values of hematological parameters between transported goat (sample, n=40) and non-transported goat (control, n=20)

Variable	Transported goat (Mean±sd)	Non-transported goat (Mean±sd)	P value
TEC (10^6/µl)	1.6±2.3	8.6±0.3	0.00
TLC (10^3/µl)	18.8±7.5	10.5±1.5	0.00
Hb (mg/dl)	8.3±1.4	8.1±0.4	0.43
PCV (%)	6.3±7.1	23.7±2.0	0.00
Lymphocyte (%)	51.6±12.2	60.7±3.5	0.00
Monocyte (%)	5.2±2.2	3.1±0.8	0.00
Neutrophil (%)	35.5±10.4	32.2±3.5	0.07
Eosinophil (%)	7.5±3.1	4.2±1.0	0.00
Basophil (%)	0.2±0.4	0±0	0.01
N:L	0.9±1.0	0.5±0.1	0.036

N:L, neutrophil lymphocyte ratio; TEC = Total Erythrocyte Count; TLC = Total Leukocyte Count; Hb= Hemoglobin; PCV= Packed Cell Volume.

Table 4.9 demonstrates a significant increase in glucose, total protein, and creatine kinase levels in transported goats compared to non-transported goats, except for phosphorus, where the difference was not significant. Additionally, calcium levels decreased in transported goats compared to non-transported goats, although this difference was not significant.

Table 4.9: Comparison of mean values of biochemical parameters between transported goat (sample, n=40) and non-transported goat (control, n=20)

Variable	Transported goat (Mean±sd)	Non-transported goat (Mean±sd)	P value
Glucose (mg/dl)	101.6±33.3	39.4±15.2	0.00
Total protein (g/l)	78.9±11.3	73.8±7.7	0.04
Calcium (mg/dl)	10.7±2.3	11.3±1.3	0.17
Phosphorus (mg/dl)	6.6±5.4	6.4±1.5	0.77
Creatine kinase (U/L)	279.7±448.4	83.9±21.8	0.01

Table 4.10: Hemato-biochemical profiles of transported goats (N=40) with high and low value percentage

Variable	Normal range*	Transported goat (Mean±sd)	Normal value (%)	High value (%)	Low value (%)
TEC (10^6/µl)	8-18	1.6±2.3	5	0	95
TLC (10^3/µl)	4-13	18.8±7.5	22.5	77.5	0
Hb (mg/dl)	8-12	8.3±1.4	65	0	35
PCV (%)	22-38	6.3±7.1	5	0	95
Lymphocyte (%)	50-70	51.6±12.2	75	0	25
Monocyte (%)	0-4	5.2±2.2	40	60	0
Neutrophil (%)	30-48	35.5±10.4	65	7.5	27.5
Eosinophil (%)	1-8	7.5±3.1	77.5	22.5	0
Basophil (%)	0-1	0.2±0.4	100	0	0
N:L	0.74 ± 0.51	0.9±1.0	0	25	75
Glucose (mg/dl)	50-75	101.6±33.3	17.5	80	2.5
Total protein (g/l)	64-70	78.9±11.3	22.5	72.5	5
Calcium (mg/dl)	8.9-11.7	10.7±2.3	72.5	17.5	10
Phosphorus (mg/dl)	4.2-9.1	6.6±5.4	45	22.5	32.5
Creatine kinase (U/L)	0.8-8.9	279.7±448.4	0	100	0

*****(*Kaneko et al., 2008; Latimer, 2011; Weiss and Wardrop, 2011); N:L, neutrophil lymphocyte ratio; TEC = Total Erythrocyte Count; TLC = Total Leukocyte Count; Hb= Hemoglobin; PCV= Packed Cell Volume.*

Table 4.10 demonstrates a 77.5% higher TLC value, an 80% higher glucose value, a 72.5% higher total protein value, and a 100% higher creatine kinase value, whereas TEC and PCV values were lower, with 95%. Hemoglobin (Hg) value was within the normal range at 65%, and among differential leukocyte counts (DLC), 75% for lymphocyte, 65% for neutrophil, 77.5% for eosinophil, and 100% for basophil values were within the normal range.

Chapter 5: Discussion

5.1 Correlation between the healthy and severe parameters of clinical signs, animal behavior, and handlers' behavior with different journey related variables

The findings presented in Tables 4.3 and 4.4 provide significant insights into the relationships between journey-related variables, animal welfare indicators, and handlers' behavior in the context of cattle, buffalo, and goats during transportation.

5.1.1 Correlation of number of animals with clinical signs, animal behavior, and handlers' behavior

The observed negative correlation between the number of animals and healthy or mild offensive parameters in cattle and buffalo highlights a significant aspect of animal transportation. Overcrowding and limited space within the vehicles can lead to stress and discomfort among animals, influencing their behavior and overall well-being. This finding emphasizes the importance of adhering to proper animal transport guidelines, ensuring sufficient space and comfort for the animals. Broom (2000) found that elevated stocking density in transport vehicles led to increased physiological stress in comparison to lower stocking density. Effective management practices, as suggested by Fisher et al. (2009), can mitigate the negative impacts of transportation on animal well-being.

5.1.2 Correlation of platform and loading place with clinical signs, animal behavior, and handlers' behavior

The orientation of the platform and the loading place both have significant impacts on animal welfare indicators. The positive correlation between an upward platform and positive handlers' behavior in cattle highlights the importance of vehicle design in facilitating better staff-animal interactions. The well-being of animals can be influenced by the conditions within transportation vehicles, emphasizing the significance of choosing a suitable vehicle for transport to minimize stress (Broom, 2003a). Similarly, the variations in loading places underscore the influence of local conditions. For instance, the South West loading area showed significant positive correlations with animal behavior in cattle, indicating potentially better handling practices or less stressful conditions in this region. Conversely, the negative correlation between clinical signs and the North East loading area in cattle suggests areas for improvement in handling techniques and animal welfare measures.

5.1.3 Correlation of journey length and stocking density with clinical signs, animal behavior, and handlers' behavior

The negative correlation between journey length and healthy or mild offensive parameters in cattle and buffalo indicates that longer journeys might lead to increased stress and discomfort among the animals. Stocking density also plays a crucial role, with increased density correlating with more severe or frequently offensive parameters in goats. These findings emphasize the need for strict implementation of regulations concerning journey duration and stocking density, ensuring animals are transported in conditions that minimize stress and discomfort. In case of goat, there was a significant negative correlation between length of journey and the severe parameters of clinical signs and the frequently offensive parameters of handlers’ behavior. This could be due to the significant positive correlation between the number of animals, and the length of the journey. In the North West loading area, the average number of goats per vehicle was 92, while in the South West area, it was 195. However, the average difference in the length of the journey for goats between the North West (400 km) and South West (425 km) loading areas was 25 km. This difference in between the number of animals, and the length of the journey could be a reason for the negative correlation between the length of journey and the severe parameters of clinical signs and the frequently offensive parameters of handlers’ behavior in goats. Extended hours of transportation in substandard vehicles can detrimentally impact animal welfare (Tarrant et al., 1992). Prolonged journeys inherently carry a higher risk of welfare issues, and certain durations can lead to problems, as emphasized by Broom (2003a). Alam et al. (2010b) documented injuries resulting from long-distance transports. Bulitta (2015) highlighted that in developing nations, animals endure extended journeys without rest, food, and water, often packed tightly and exposed to sunburn. When animals are densely packed, there is a risk that those that slip and fall may struggle to rise and could potentially be crushed, as highlighted by Strappini et al. (2009).

5.2 Correlation between clinical signs, animal behavior, and handlers' behavior

The correlations illustrated in Figure 4.2 and 4.3 offer valuable insights into the intricate interconnections among clinical signs, animal behavior, and handlers' behavior during transportation, specifically focusing on cattle and buffalo. Understanding these relationships is fundamental for enhancing animal welfare standards in the context of transportation practices.

The positive correlation observed between clinical signs and animal behavior in both cattle and buffalo indicates a direct relationship between the animals' physiological state and their behavioral responses. Stress and discomfort, often manifested through clinical signs, can trigger altered behaviors such as restlessness, aggression, or fear. This finding aligns with existing literature in animal welfare, emphasizing the importance of recognizing behavioral changes as potential indicators of an animal's well-being or distress. The key signs that an animal struggles with handling and transportation involve alterations in behavior, indicating whether certain situations are perceived as unpleasant or not, as stated by Bulitta (2015).

Moreover, the positive correlation between animal behavior and handlers' behavior highlights the significant role of human-animal interactions. Gentle handling and positive interactions with staff can alleviate animals' stress, creating a calmer environment during transportation. While this correlation was significant in both cattle and buffalo, the non-significant positive correlation between clinical signs and handlers' behavior in cattle suggests a trend, though not statistically strong. This trend implies that positive staff interactions might slightly mitigate the physiological stress responses in cattle, although not significantly. A weak bond between humans and animals can lead to stress and accidents for both parties and requires enhancement (Coleman et al., 2000). Similarly, it's widely recognized that the attitudes of livestock handlers toward their animals are closely linked to their conduct when dealing with the animals, and harsh handling can detrimentally impact animal welfare and heighten the animals' fear of humans, as indicated by Ceballos et al. (2018).

The findings also highlight species-specific differences in how cattle and buffalo respond to transportation stressors. In buffalo, both clinical signs and animal behavior exhibited significant positive correlations with handlers' behavior. This suggests that in buffalo, stress indicators and behavioral responses are more visibly influenced by the way staff interact with them. Understanding these species-specific variations is crucial for adapting handling practices, as different animals may respond differently to similar stimuli.

5.3 Differences between clinical signs, animal behavior, and handlers' behavior

5.3.1 Differences between clinical signs

Clinical signs provide valuable insights into animal welfare throughout the transportation process. The prevalence of nasal discharge, ocular discharge, and foamy mouth highlights the physiological stress experienced, particularly among cattle and buffalo. The differences in injury types further emphasize the need for specific attention to injury prevention measures, especially in buffalo, where old injuries and lacerations were notably higher. Fresh bleeding, another concerning indicator of stress, was more prevalent in buffalo than in cattle. These observations emphasize the necessity for customized handling and transport protocols for different species. Understanding the specific signs that each species manifests under stress is crucial for early detection and appropriate intervention. The prevalence of old injuries, lacerations, and fresh bleeding in buffalo raises concerns about the conditions in which these animals are transported. It suggests that buffalo might be more susceptible to injuries during transportation, possibly due to their size, behavior, or handling techniques. One of the main reasons for observing more clinical signs in buffalo compared to other species is that most of the buffalo were transported from the border area of Bangladesh, where they may get injured during border crossing. Cattle displayed a significantly higher proportion of animals with good cleanliness compared to buffalo. Addressing the underlying causes of these injuries, such as inappropriate loading and unloading procedures or inadequate vehicle design, is crucial to minimize the risk of harm to the animals. In conclusion, the diverse clinical signs observed among goats, cattle, and buffalo highlight the complex nature of animal responses to transportation stress. Alam et al. (2018) documented a notable increase in nasal discharge among cattle following transportation, a finding that supports the results observed in previous studies conducted by Ishizaki et al. (2005) and Mitchell et al. (2008). The heightened incidence of nasal discharge in transported cattle could be attributed to various factors, including the infiltration of microorganisms into the upper respiratory tract (Lin et al., 2000; Storz et al., 2000; Knowles et al., 2014). This infiltration is a consequence of immune suppression due to transportation-induced stress noted by Buckham Sporer et al. (2007). Additionally, factors such as the intrusion of dust, highlighted by Onishi et al. (2012), and heat stress, suggested by Bernabucci et al. (2014), might also contribute to this phenomenon. Studies conducted in Bangladesh, including those by Alam et al. (2010c) and Kober et al. (2014), have reported elevated instances of physical injuries resulting from transportation. Similarly, other researchers such as Minka and Ayo (2007a), Gregory (2008), Rakib et al. (2016), and Alam et al. (2018) have documented an increased frequency of injuries among cattle after transportation.

5.3.2 Differences between animal behavior, and handlers' behavior

The data presented in Tables 4.6 and 4.7 shed light on the behavioral responses of transported animals and the conduct of transport staff during unloading. Understanding these behaviors is crucial for enhancing animal welfare standards in the transportation industry.

The significant differences in animal behavior among goats, cattle, and buffalo emphasize the need for species-specific transport protocols. Goats displayed a higher incidence of jumping (35.6%) and falling (18.4%), indicating their agile nature and potential restlessness during transportation. The absence of a platform for goats may contribute to the higher incidence of jumping and falling. Cattle and buffalo, on the other hand, were more prone to refusing to move, slipping, and running away. A higher incidence of jumping was observed in cattle and buffalo when they were unloaded using a downward platform, compared to other platforms. Adapting transport practices to accommodate these species-specific behaviors is essential. The observed behaviors, such as refusing to move, falling, and running away, are indicative of stress and fear in animals. Stress during transportation can lead to injuries, exhaustion, and compromised animal welfare. During the process of loading and unloading, Bravo et al. (2020) documented occurrences such as slips, falls, jumps, balks, turns, vocalizations, mounts, aggressions, and defecation/urination. Similarly, Lindahl et al. (2016) noted behavioral events including falls, aggression/fights, slips, jumps, balks, reversing, freezing, running, and vocalizations. Messori et al. (2015) observed that a majority of the flocks experienced slipping upon unloading, and in one-third of the cases, the flocks displayed hesitation to move.

The behavior of transport staff significantly influences the animals' experiences. Cattle transporters demonstrated notably higher levels of friendly behavior (40.5±8.6) compared to other species, indicating a positive interaction between staff and animals. Conversely, the incidence of rude behaviors such as beating, slapping, pushing, and tail twisting/lifting, especially in cattle and buffalo, raises concerns about the well-being of the animals. The absence of a training program for transport staff on gentle animal handling and stress reduction techniques during transportation, coupled with their lack of knowledge about animal welfare and proper handling, may lead to a high frequency of rude behavior. The presence of rude behaviors, especially in the form of beating and tail twisting/lifting, raises ethical concerns. The most popular tool used to drive calves during loading and unloading was a wooden stick, according to Bravo et al. (2020), who also documented the incidence of forbidden behaviors such as hitting, poking, kicking, and tail bending or tugging. A research on cattle handlers in slaughterhouses in Chile (Strappini et al., 2013) examined the unpleasant tactile interactions that handlers utilized, such as poking, striking, and tail twisting (Leon et al., 2020). Tail twisting is deeply embedded in the cattle management cultures of various countries, including Colombia (Herrán et al., 2017; Romero et al., 2017), Chile (Strappini et al., 2013), and Bangladesh (Alam et al., 2010b). The harmful impact of this practice on cattle handling likely depends on the force exerted by the handler. Consequently, merely observing this interaction isn't sufficient to differentiate between forceful and gentle tail twisting (Pajor et al., 2000). Difficulties arise in animal handling, leading to increased stress levels, especially when handlers lack proper techniques, which can significantly affect their behavior in their daily work routines (Ceballos et al., 2018).

5.4 Profile of blood physiology of transported goats

5.4.1 Hematological profile

The hematological parameters presented in Table 4.8 offer valuable insights into the physiological effects of transportation on goats. These findings provide a basis for understanding the impact of transportation stress on the animal's immune system and overall health.

Transportation stress is reflected in the significant decrease in total erythrocyte count (TEC) and packed cell volume (PCV) in transported goats. This reduction indicates anemia and decreased oxygen-carrying capacity, potentially affecting the animal's overall health and vitality. Anemia could result from stress-related factors such as dehydration, reduced feed intake, or increased metabolic demands during transportation. Mitchell et al. (2008), Ambore et al. (2009), and Hulbert et al. (2011)

proposed that transportation results in an increase in PCV, TEC, and hemoglobin levels, leading to haemoconcentration. In contrast, earlier studies indicated that hemoglobin (Hb) and total erythrocyte count (TEC) values in cattle remained unchanged after transportation. This lack of change could be attributed to short-distance journeys (<10 hours) and the availability of adequate water and space during transportation, as observed in studies by Ishiwata et al. (2008) and Mitchell et al. (2008).

The substantial increase in TLC (total leukocyte count) in transported goats suggests a heightened immune response, likely due to stress. This elevation is attributed to the rise in monocytes, eosinophils, and basophils, indicating an activated immune system. Monocytes are associated with the body's defense against pathogens and inflammation, whereas eosinophils and basophils are involved in allergic and parasitic responses. The increase in these cell types suggests an immune response to stressors encountered during transportation. The results align with previous research conducted in Bangladesh (Rakib et al., 2016; Alam et al., 2018) and neighboring countries (Ishizaki et al., 2005). Consistent findings have also been reported by other researchers (Hulbert et al., 2011; Stockman et al., 2013). Some studies have shown no significant change in total leukocyte count (TLC) after short-duration transportation (4 hours) (Hulbert et al., 2011). However, in our study, the increase in white blood cell proliferation could be associated with the stimulating impact of glucocorticoids during prolonged transportation (Weber et al., 2006).

The decrease in lymphocyte percentage in transported goats suggests immunosuppression, rendering them more susceptible to infections. Lymphocytes are vital components of the immune system, and their reduction indicates a compromised immune response. Stress-related factors can suppress lymphocyte production and activity, impacting the animal's ability to effectively fight infections. The results are consistent with findings from various researchers (Buckham Sporer et al., 2007; Mitchell et al., 2008; Hulbert et al., 2011; Stockman et al., 2013). The decline in lymphocyte count observed in our study can be attributed to the fact that lymphocytes typically receive stimulation from glucocorticoid and adrenergic receptors on their surfaces (Preisler et al., 2000). However, under stress conditions, these receptors undergo downregulation, as suggested by Burton et al. (2005). In contrast, Mitchell et al. (2008) reported no changes in lymphocyte counts in cattle before and after transportation, possibly due to the short distance of travel and effective transportation management practices.

The higher neutrophil-lymphocyte ratio (N:L) in transported goats further supports the stress-induced immune response. An elevated N:L ratio is often considered an indicator of physiological stress, with neutrophilia reflecting acute stress while lymphopenia indicates compromised immune function. The significant increase in the N:L ratio indicates a substantial stress response in transported goats, potentially compromising their immune system's efficiency. This discovery aligns with the research findings of multiple researchers (Hulbert et al., 2011; Stockman et al., 2013).

The alterations in hematological parameters highlight the physiological stress experienced by goats during transportation. Stressors such as noise, vibration, temperature fluctuations, and social disruption can lead to these changes. In conclusion, the hematological changes observed in transported goats emphasize the significance of considering the stress-induced immune response in animal welfare assessments.

5.4.2 Biochemical profile

The biochemical parameters outlined in Table 4.9 offer valuable insights into the physiological impact of transportation on goats. These results provide a basis for understanding the metabolic and biochemical changes induced by transportation stress.

The significant increase in glucose levels among transported goats indicates a stress-induced metabolic response. Stress induces the secretion of stress hormones such as cortisol, which can result in increased blood glucose levels through processes like gluconeogenesis. Prolonged elevation in glucose can have various effects, including increased energy consumption and altered metabolism. While a short-term increase is natural during stressful events, chronic stress could have long-term implications for the animal's metabolic health. Transportation stress led to elevated plasma glucose levels in goats, as documented by Kannan et al. (2000) and Kannan et al. (2003). Similarly, research conducted by Zulkifli et al. (2010), Hulbert et al. (2011), and Panchal et al. (2022) reported a notable rise in serum glucose concentration in transported cattle. In contrast, Ishiwata et al. (2008) reported a significant decrease in serum glucose concentration in cattle after transportation. Alam et al. (2018) recorded a non-significant reduction in serum glucose concentration in transported cattle, consistent with findings by Stockman et al. (2013).

The increase in total protein levels suggests a potential stress response. Stress-related inflammation and tissue damage can lead to an increase in acute-phase proteins, contributing to elevated total protein levels. While a moderate increase might be part of the stress response, chronic elevation could indicate prolonged stress, which might impact the animal's overall health and immune function. Our findings align with the research results of Okoruwa (2014) in goats, Panchal et al. (2022) in goats, and Alam et al. (2018) in cattle. Similar patterns were observed in several earlier studies (Averós et al., 2008; Averós et al., 2009). The promotion of protein metabolism due to elevated T_3 secretion during transportation might account for the increased TP levels. Additionally, post-transport dehydration can result in water loss from the blood and haemo-concentration, which could also contribute to the rise in TP levels, as suggested by Friend (2000).

The significant elevation in creatine kinase levels in transported goats highlights muscular stress or damage. CK is an enzyme found in muscles, and increased levels often indicate muscle injury or stress. During transportation, animals may experience physical strain due to movement within the vehicle or while loading and unloading. High CK levels underscore the need for careful handling and appropriate transportation practices to minimize physical stress on the animals. Typically, creatine kinase (CK) levels rise during transportation, as noted in studies by Grigor et al. (2001), Fisher et al. (2014), and Marcato et al. (2020). Alam et al. (2018) observed a significant elevation in CK concentrations in cattle immediately after transportation. This elevation in serum CK concentration could be attributed to muscle breakdown resulting from prolonged muscle activity during transportation, as suggested by Buckham Sporer et al. (2008).

While calcium levels decreased in transported goats, the difference was not statistically significant. Calcium is vital for various physiological functions, including muscle function and nerve transmission. A decrease, even if not significant, might affect these functions, emphasizing the importance of monitoring calcium levels during transportation. Phosphorus levels remained relatively stable, indicating that transportation might not significantly impact phosphorus metabolism in healthy goats.

Elevated levels of serum calcium and phosphorus were observed following transportation in cattle by Alam et al. (2018) and in goats by Panchal et al. (2022). This increase could be attributed to hyperparathyroidism, which maintains blood calcium concentration during long-distance transportation, as suggested by Chakera et al. (2012). Ayo et al. (2009) also noted a rise in serum calcium concentration and a decrease in serum phosphorus concentration in transported goats.

The changes in biochemical parameters highlight the physiological stress experienced by goats during transportation. Prolonged stress can result in detrimental effects on health, well-being, and overall performance. In conclusion, the alterations in glucose, total protein, and creatine kinase levels indicate a stress response in transported goats.

Chapter 6: Conclusions

The findings highlighted the crucial importance of adhering to proper animal transportation regulations by providing insights into the intricate interplay between animal behavior, physiological responses, and transport staffs' interactions during unloading of transported animal. Insufficient space stress and journey length negatively impact healthy parameters in cattle and buffalo, emphasizing the urgent need for compliance with stocking density guidelines and strict regulations on travel duration to minimize transportation-related stress. Customized handling methods are crucial due to varied behavioral responses among goats, cattle, and buffalo during transportation, emphasizing the need for tailored approaches. Positive staff conduct is associated with reduced transportation-related stress, highlighting the importance of compassionate interactions for animal welfare. The physiological changes observed in transported goats underscore the necessity of continuous monitoring to minimize the negative impact of transportation stress on their health and welfare, particularly evident in hematological and biochemical factors.

Chapter 7: Recommendations

The investigation underscores the importance of implementing and enforcing precise laws for animal transportation to mitigate stress and suffering. Staff training in gentle handling techniques and stress reduction strategies is recommended to foster positive human-animal interactions. Vehicle designs should be tailored to accommodate different species' behaviors, prioritizing safety and accident prevention. Utilizing suitable, non-slip platforms during unloading, especially for goats, is essential to prevent injury. Collaborative efforts among regulatory bodies, transport providers, and animal welfare organizations are crucial for establishing a consistent framework that prioritizes animal well-being. In the future, research could be conducted by observing animals during loading, transportation, and unloading to ascertain the real effects of transportation on animals.

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Annex-I: Questionnaire

Name of market:	Date:
Address:	
Starting time:	Ending time:

Animal loading place: Chuadanga/Jessore/Kushtia/Pabna/Rajshahi/Rangpur/ Chapainawabganj/others………….

Vehicle information:

Serial number:	Total animal:
Vehicle type: Truck/pickup/others……………	
Floor/surface area (ft.)	Length (ft.):
	Width (ft.):
	Height (ft.):
Stocking density (animal/sq. ft.):	

Animal status:

Species: Cattle/Buffalo/Goat/others………..	Origin: Local/imported
Breed:	
Animal type: Bull/Bullock/Cow/Buck/Wether/Doe/others……………	
Sex	Male (No……….)
	Female (No………)
Arrangement of animals in the vehicle	Direction to travel
	Opposite to travel
	Perpendicular
	Mixed
Body conformation of animals (cattle & buffalo)	Small (<200 kg):
	Medium (200-400 kg):
	Large (>400 kg):

Transport related information:

<table>
<tr><td colspan="2">Loading place (upazila & district):</td></tr>
<tr><td>Loading time:</td><td>Unloading time:</td></tr>
<tr><td>Temperature (during unloading):</td><td>Humidity (during unloading):</td></tr>
<tr><td rowspan="4">Platform used during unloading</td><td>No platform</td></tr>
<tr><td>Level</td></tr>
<tr><td>Downward</td></tr>
<tr><td>Upward</td></tr>
<tr><td>Duration of journey (hr.):</td><td>Length of journey (km):</td></tr>
<tr><td colspan="2">Nature of journey: Continuous/Break for animal/others………..</td></tr>
<tr><td rowspan="2">Feed provided during journey</td><td>Yes</td></tr>
<tr><td>No</td></tr>
<tr><td rowspan="2">Water provided during journey</td><td>Yes</td></tr>
<tr><td>No</td></tr>
</table>

Behavior of animals during unloading (number):

Refuse to move:
Jump:
Fall:
Slip:
Run away:
Confusion:

Attitude and behavior of transport staff during unloading (number):

<table>
<tr><td colspan="2">Friendly:</td></tr>
<tr><td rowspan="6">Rude</td><td>Beating:</td></tr>
<tr><td>Slapping:</td></tr>
<tr><td>Pushing:</td></tr>
<tr><td>Hanging by rope:</td></tr>
<tr><td>Dragging by ear:</td></tr>
<tr><td>Tail twisting and lifting:</td></tr>
</table>

Clinical signs observed in the animal during unloading (number):

Nasal discharge:	Ocular discharge:
Foamy mouth:	Lameness:
Fresh bleeding:	Dead:
Recumbent:	
Injury type	Old:
	Fresh:
Injury/wound	Abrasion:
	Laceration:
	Others:
Abdomen state	Full:
	Hollow:
Cleanliness of body/skin	Good (dirt present in <20% body area):
	Fair (dirt in 20-40%) of body area):
	Poor (dirt in >40% of body area):

Practice used by transport staff to stand up the recumbent animal (number):

Shouting:
Beating by wood/rope/plastic pipe/metal:
Slapping:
Lifting of or dragging by tail:
Tail biting:
Tail twisting:
Breathing inhibition:

Table: Definition of different clinical signs observed during unloading of animals

Clinical sign	Definition
Nasal discharge	It refers to abnormal fluid coming from animals' nose, often indicating underlying health issues such as infections, allergies, or respiratory problems.
Ocular discharge	It refers to abnormal fluid coming from animals' eyes, which can result from issues such as infections, allergies, trauma, blocked tear ducts, or foreign bodies.
Foamy mouth	It refers to the presence of frothy saliva or foam around the mouth of the animal.
Injury	
Old	An old injury in animals refers to a previously sustained trauma or damage to tissues, characterized by features such as scar tissue, changes in color, or altered function.
Fresh	A fresh injury in animals refers to a recent or newly acquired trauma or damage to tissues, specifically on the epidermal layer of the skin, characterized by inflammatory signs such as redness, swelling, heat, and pain.
Abrasion	Abrasion in animals is a superficial wound caused by friction, scraping, or rubbing of the skin surface, often involving the removal of the outermost layer of skin (epidermis), leading to mild bleeding, pain, and the absence of deep tissue involvement.
Laceration	A laceration in animals is a more severe type of injury characterized by a torn or jagged wound with irregular edges, involving deeper layers of skin and potentially extending into underlying tissues, such as muscles or tendons.
Fresh Bleeding	Fresh bleeding in animals refers to the recent release of red blood from a wound.
Lameness	Lameness in animals refers to a condition where an animal experiences difficulty or abnormality in its gait or ability to walk, often due to pain, injury, or musculoskeletal issues.
Abdomen	
Full	Full abdomen typically refers to an enlarged or distended belly.

Hollow	Hollow abdomen typically indicates a lean or undernourished state, characterized by insufficient body fat or muscle mass, resulting in a visibly concave or sunken appearance in the abdominal region.
Cleanliness of the body or skin	
Good	The presence of dirt on less than 20% of the body area.
Fair	The presence of dirt on 20-40% of the body area.
Poor	The presence of dirt on more than 40% of body area.

Table: Definition of different behaviors of transport staffs observed during unloading of animals

Observed behavior	**Definition**
Friendly	Friendly behavior in animals refers to positive, amicable actions, including expressions of affection, approachability, and non-aggressive social interactions.
Rude	
Beating	Beating in animals refers to aggressive actions involving repeated strikes or blows, often using objects such as wood, rope, plastic pipes, metal, etc.
Slapping	Slapping in animals involves striking with an open hand and can occur during aggressive encounters.
Pushing	Pushing of animals by handlers as a rude behavior refers to the forceful and disrespectful use of physical pressure to move or control an animal.
Hanging by rope	Tighten the animal's head with a rope and then bring it down from the vehicle to the ground by hanging it with the rope.
Dragging by ear	Dragging an animal by the ear refers to an inhumane practice of moving or pulling the animal by gripping its ear, potentially causing pain and distress.
Tail twisting and lifting	Tail twisting and lifting in animals by handlers are considered rude behaviors, involving the forceful rotation or manipulation of the tail, causing pain and distress to the animal.

Annex-II: Biochemical assay

1. Glucose assay

Assay principle

The principles outcome of glucose is based on the principle of competitive bindings between glucose in the test specimen and GOD-PAP reagent of glucose. The glucose is determined after enzymatic oxidation in the presence of glucose oxidase. The formed hydrogen peroxide reacts under catalysis of peroxidase with phenol and 4-aminophenazone to a red-violet quinoneimine dye as indicator.

Assay reaction

Glucose + O_2 + H_2O = Gluconic acid + H_2O_2

2 H_2O_2 + 4-aminophenazone + Phenol = Quinoneinine + 4 H_2O

Materials and reagents

1. Serum sample: 10 µL
2. Glucose standards: 10 µL
3. Glucose conjugate reagent: 1000 µL
4. Precision pipettes: 10 µL and 1000 µL
5. Eppendorf tube, eppendorf tube holder, automated humalyzer (Humalyzer 3000, Germany), disposable pipette tips (yellow and blue), distilled water, 70% alcohol, paper towel, cotton and gloves

Procedure:

The hands were gloved and then sterilized by the 70% alcohol. The bench was sterilized with the help of 70% alcohol and paper towel. The humalyzer were switched on and the program was set at 545 nm. The sterile eppendorf tube was taken. Then 10 µL of Glucose standards was taken in an eppendorf tube and 10 µL of sample serums were taken in each eppendorf tube. 1000 µL of Glucose conjugate reagent was then added to each eppendorf tube. The eppendorf tube was then incubated at 37ºC for 10 minutes. Glucose standards with conjugate reagent were examined first for determined of the standard value. Then all eppendorf tubes containing sample serum with Glucose conjugate reagent was examined by automated humalyzer and the reading was taken. The standard value was used as a compared tool.

2. Total protein assay

Colorimetric spectrophotometric methods were used for determination of total protein.

Assay principle

The principles outcome of total protein is based on the principle of competitive bindings between cupric ions react with protein in alkaline solution to form a purple complex. The absorbance of this complex is proportional to the protein concentration in the sample.

Assay reaction

$$Cu^{2+} + \text{Protein} \longrightarrow \text{Purple Complex}$$

Materials and reagents

1. Serum sample: 20 μL
2. Total protein standards: 20 μL
3. Total protein conjugate reagent: 1000 μL
4. Precision pipettes: 20 μL and 1000 μL
5. Eppendorf tube, eppendorf tube holder, automated humalyzer (Humalyzer 3000, Germany), disposable pipette tips (yellow and blue), distilled water, 70% alcholol, paper towel, cotton and gloves

Procedure

The hands were gloved and then sterilized by the 70% alcohol. The bench was sterilized with the help of 70% alcohol and paper towel. The humalyzer were switched on and the program was set at 545 nm. This was a photometric colorimetric test for total proteins are called Biuret method. The sterile eppendorf tube was taken. Then 20 μL of total protein standards was taken in an eppendorf tube and 20 μL of sample serums were taken in each eppendorf tube. 1000 μL of total protein conjugate reagent was then added to each eppendorf tube. The eppendorf tube was then incubated at 37ºC for 10 minutes. Total protein standards with conjugate reagent were examined first for determined of the standard value. Finally, all eppendorf tubes containing sample serum with TP conjugate reagent was examined by automated humalyzer and the reading was taken. Standard value was used as a compared tool.

3. Calcium (Ca) assay

Assay principle

The principle outcome of calcium is based on the principle of competitive bindings between Ca and Ca reagent which is a Colorimetric method that is O-Cresolphthalein complexone, without depolarization. Calcium ion forms a violet complex with O-Cresolphthalein complexone in an alkaline medium. Intensity of the colour formed is directly proportional to the amount of calcium present in the sample.

Assay reaction

Ca^{2+} + O-Cresolphthalein complexone $\longrightarrow$ Violet Complex

Materials and reagents

1. Serum sample: 25 μL
2. Calcium (Ca) standards: 25 μL
3. Calcium (Ca) conjugate reagent: 500 μL
4. Precision pipettes: 25 μL and 500 μL
5. Eppendorf tube, eppendorf tube holder, automated humalyzer (Humalyzer 3000, Germany), disposable pipette tips (yellow and blue), distilled water, 70% alcholol, paper towel, cotton and gloves

Procedure

The hands were gloved and then sterilized by the 70% alcohol. The bench was sterilized with the help of 70% alcohol and paper towel. The humalyzer were switched on and the program was set at 580 nm. The sterile eppendorf tubes were taken. Then 25 μL of Ca standards was taken in an eppendorf tube and 25 μL of sample serums were taken in each eppendorf tube. 500 µL R_1 and 500 µL R_2 of Ca conjugate reagent was then added to each eppendorf tube. The eppendorf tube was then incubated at 37ºC for 5 minutes. Ca standards with conjugate reagent were examined first for determined of the standard value. Then all eppendorf tubes containing sample serum with Ca conjugate reagent was examined by automated Humalyzer and the reading was taken. The standard value was used as a compared tool.

4. Phosphorus (P) assay

Assay principle

The principles outcome of Phosphorus is based on the principle of competitive bindings between Phosphorus and Phosphorus reagent which is a Photometric UV Test for the determination of Phosphorus. Phosphorus reacts with molybdate in strong acidic medium to form a complex. The absorbance of this complex in the near UV is directly proportional to the phosphate concentration.

Assay reaction

$$PO_4^{3-} + H^+ + (NH_4)_6Mo_7O_{24} \longrightarrow \text{Phosphomolybdic complex}$$

Materials and reagents

1. Serum sample: 10 µL
2. Phosphorus (P) standards: 10 µL
3. Phosphorus (P) conjugate reagent: 1000 µL
4. Precision pipettes: 10 µL and 1000 µL
5. Eppendorf tube, eppendorf tube holder, automated humalyzer (Humalyzer 3000, Germany), disposable pipette tips (yellow and blue), distilled water, 70% alcholol, paper towel, cotton and gloves

Procedure

The hands were gloved and then sterilized by the 70% alcohol. The bench was sterilized with the help of 70% alcohol and paper towel. The humalyzer were switched on and the program was set at 340 nm. The sterile eppendorf tubes were taken. Then 10 µL of

Phosphorus standards was taken in an eppendorf tube and 10 μL of sample serums were taken in each eppendorf tube. 1000 μL of Phosphorus conjugate reagent was then added to each eppendorf tube. The eppendorf tube was then incubated at 37ºC for 5 minutes. Phosphorus standards with conjugate reagent were examined first for determined of the standard value. Then all eppendorf tubes containing sample serum with Phosphorus conjugate reagent was examined by automated Humalyzer and the reading was taken. The standard value was used as a compared tool.

5. Creatine kinase assay

Assay principle

This study is an enzymatic colorimetric method. Creatine kinase catalyzes the reversible phosphorylation of creatine by ATP. Because phosphocreatine (creatine phosphate) has a significantly higher energy than ATP, the equilibrium is largely shifted into the reverse direction. At the presence of hexokinase, this ATP is then bind with D-Glucose and broken down in to ADP and G-6-Phosphate. The G-6-Phosphate is then bind with NADP in the presence of D-glucose-6-phosphate-dehydrogenase and produce 6-Phosphogluconate and NADPH and release a proton. The course of the reaction is then monitored spectrophotometrically by measuring the conversion of NADP to NADPH, which is done by following the increase in absorbance at 340 nm.

Assay reaction

At the presence of Creatine Kinase,

Creatine phosphate + ADP ⟶ Creatine + ATP

Then at the presence of Hexokinase,

D-Glucose + ATP ⟶ ADP + G-6-Phosphate

Further, at the presence of D-glucose-6-phosphate-dehydrogenase,

G-6-Phosphate + NADP ⟶ 6-Phosphogluconate + NADPH + H^+

Materials and reagents

1) Serum sample: 20 µL
2) Substrate-Enzyme (di-Adenosine-5-pentaphosphate, Creatine Phosphate, N-acetyle-L-cystein, ADP, NADP+ , AMP, Glucose-6-phosphate-dehydrogenase and Hexokinase) from SPINREACT®
3) Buffer reagent (Imidazole acetate, EDTA Na2, Magnesium Acetate and Glucose) from SPINREACT®
4) Pipette: 20 µL and 1000 µL pipette
5) Eppendorf tube, eppendorf tube rack, automated humalyzer (Humalyzer 3000, Germany), disposable pipette tips (yellow and blue), distilled water, 70% alcohol, paper towel, cotton and gloves

Procedure

The hands were gloved and then sterilized by the 70% alcohol. The bench was sterilized with the help of 70% alcohol and paper towel. The humalyzer were switched on and the program was set at 340 nm. The factor (calibrator activity) was added to be multiplied with the CK activity. Here the factor was 8095. The temperature conversion factor at 37°C was 100. 15 mL of buffer reagent was added with one tablet of substrate-enzyme, mixed thoroughly and vortexed. Then 1 mL of this mixture reagent was taken in an eppendorf tube and 20 µL of sample was taken in that same eppendorf tube. The complex was vortexed and then ran under 340 nm in the humalyzer. All the samples were tested by the same manner.

Biography

Md. Ismail, son of Abu Nasar Md. Idris Kutubi and Zobaida Begum, was born in Cox's Bazar on 1st June, 1997. He passed the Secondary School Certificate examination from Chattogram Government Muslim High School, Chattogram, in 2013, followed by the Higher Secondary Certificate examination from Hazera-Taju University College, Chattogram, in 2015. He obtained a Doctor of Veterinary Medicine (DVM) degree from Chattogram Veterinary and Animal Sciences University (CVASU), Chattogram, Bangladesh, in 2020. Currently, he is a candidate for the degree of Master of Science (MS) in Physiology under the Department of Physiology, Biochemistry and Pharmacology, Faculty of Veterinary Medicine, CVASU. He is strongly interested in working in the field of animal welfare.