



Assessment of transport stress on cattle travelling a long distance (≈ 648 km), from Jessore (Indian border) to Chittagong, Bangladesh

Mahabub Alam,¹ Md Hasanuzzaman,¹ Mohammad Mahmudul Hassan,¹ Tofazzal Md Rakib,¹ Md Emran Hossain,¹ Md Harun Rashid,¹ Md Abu Sayeed,¹ Lindsay B Philips,² Md Ahasanul Hoque¹

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ABSTRACT

The effect of long-distance transport on cattle health has not frequently been studied in Bangladesh. The current study investigated the health conditions, and the extent and pattern of cattle injuries, along with haemato-biochemical and hormonal changes, before and after long-distance transportation (≈ 648 km) from the market of origin to the market of destination. A total of 100 adult cattle were selected at the Benapole live cattle market, Bangladesh, for physical examination before and after transportation. Fifty of these cattle were randomly selected for additional haemato-biochemical evaluation just before the start of transportation (0 hour), immediately after arrival at the destination market (13.8 \pm 0.9 hours after the start of transportation) and 24 hours after arrival at the destination market. The external health conditions and injuries were assessed. Animals were fasting in the vehicle during transportation and provided only with paddy straw and water before sale at the destination market. Before and after transportation, the overall frequency of cattle injuries varied significantly (26 per cent before v 47 per cent after transportation; $P < 0.001$). Cattle health conditions diverged significantly (such as nasal discharge: 15 per cent v 28 per cent; $P = 0.03$). The values of haemoglobin ($P = 0.01$), total erythrocyte count ($P = 0.001$), total leucocyte count ($P < 0.001$), lymphocyte ($P = 0.005$), neutrophil ($P = 0.01$) and eosinophil ($P = 0.01$) varied significantly. The values of serum total protein ($P = 0.006$), creatine kinase ($P < 0.001$), triglyceride ($P = 0.04$), calcium ($P = 0.003$), phosphorus ($P < 0.001$) and alkaline phosphatase ($P = 0.04$) significantly differed. The overall findings indicate a high degree of transport stress and poor animal welfare.

INTRODUCTION

In today's modern society, transport of live-stock is an integral part of farming. Live animals may be moved due to a change of ownership from one farm to another, a change in the rearing areas, and for marketing from short-distance or long-distance sale yard, live cattle market, abattoir, feedlot or pre-export assembly depot.¹

Transport stress is a complex issue. Many things factor into transportation stress to

include transport management, climatic factors (temperature, humidity, gases and so on), handling methods and facilities, feed and water deprivation, and so on.¹ An animal's response to the effects of transportation stress creates a complex interaction between neurons and hormones. The results of such interactions are manifested clinically (Minka and Ayo)² by changes in physical (external changes), haematological, biochemical and hormonal parameters (internal changes) of the body.³ In general, the biochemical changes due to transportation stress include a decrease in glucose concentrations, and an increase in non-esterified free fatty acid, muscle enzymes (such as creatine kinase),^{4 5} packed cell volume (PCV)⁶ and serum protein.^{3 4} Due to activation of the hypothalamic-pituitary-adrenal axis (HPA),¹ researchers have also reported marked changes in cortisol and catecholamine in different stages of transportation.^{6 7}

Considering the immune response, transport stress results in increases in the number of total white blood cells, neutrophils, eosinophils and mononuclear cells in particular. However, transport stress results in decreased lymphocyte counts.^{8 9} These changes in blood parameters may be useful biomarkers in estimating the degree of transport stress.¹⁰ Transport stress has a negative effect on production and reproductive performance¹¹; for example, it causes liveweight loss that varies from 3 to 12 per cent depending on the duration and conditions of a journey.¹²

The transportation of livestock in Bangladesh is mainly performed by road vehicles. Bangladesh is a tropical country where temperature (average 30°C–40°C) and humidity (average 60–85 per cent) become higher during the summer season. Most days



¹Faculty of Veterinary Medicine, Chittagong Veterinary and Animal Sciences University, Chittagong, Bangladesh

²Cummings School of Veterinary Medicine at Tufts University, Boston, Massachusetts, USA

Correspondence to

Professor Md Ahasanul Hoque; md.hoque@my.jcu.edu.au

are very hot and the UV exposure is high. Animal transporters attempt to move animals under adverse weather conditions and this can be detrimental to the animals. Various cruel practices such as rough handling, high stocking density, transport in high temperature, humidity and so on are different forms of welfare violations that have been observed, and these practices increase the risk of muscle injury and fatigue.¹³ In a nutshell, transportation stress results in multidirectional effects that include body injuries, bodyweight shrinkage and degradation of meat quality.¹⁴ Transportation can also decrease immunity, increase infectious diseases susceptibility and eventually result in the death of an animal. Therefore, the current study investigated the health conditions, and the extent and pattern of cattle injuries, along with haemato-biochemical and hormonal changes, before and after long-distance transportation (≈ 648 km) from the market of origin to the market of destination.

MATERIALS AND METHODS

Live Cattle Market (LCM) and the study period

Two LCMs in Bangladesh were purposively selected for the study: (1) Benapole LCM (market of origin; the market from where the transport of cattle was started), which is situated in Jessore District ($23^{\circ}11'39.8''$ N and $89^{\circ}04'02.5''$ E), about 648 km from Chittagong; and (2) Sagorika LCM (destination market; the market where the travel was ended and cattle were unloaded), which is located within the Chittagong metropolitan city ($22^{\circ}21'31.4''$ N and $91^{\circ}46'43.1''$ E) (Fig 1). The Benapole

LCM was chosen because of its proximity to the Indian border with southern Bangladesh and the potential for trade of different exotic (imported from India) cattle breeds (*Bos indicus*) like Hariana, Hallikar, Gir, Ongole and Tharparkar. During the peak season (August to September), just before Eid-ul-Adha, a big festival for the Muslim community in Bangladesh when people sacrifice animals to satisfy God, between 20,000 and 25,000 cattle are legally or illegally imported each day from India to Bangladesh.¹⁵ Haat days, or trading days, at Benapole LCM run on Saturday and Tuesday of each week. This market is the largest of its kind in the south-western region of Bangladesh. On any given haat day, around 15,000–20,000 cattle are kept on the market, while on average nearly 10,000 cattle are sold.¹⁶

Sagorika LCM trades local cattle as well as Indian cattle, which are transported from the LCM of Benapole in Jessore. The usual size for trading in this market during the peak season (August to September, just before Eid-ul-Adha) has been estimated to be 15,000–20,000 per day.¹⁷

Although the distance between the markets is quite long for transporting cattle, this long-standing traditional practice of cattle trading exists because of better market price achieved through sales in the Chittagong region. The animals were not immediately sold out but usually stocked at the destination market for two to three days before the sale. The present study was conducted between July and December 2015, where the fieldwork was performed between August and September 2015, which corresponded to the peak season of cattle trade

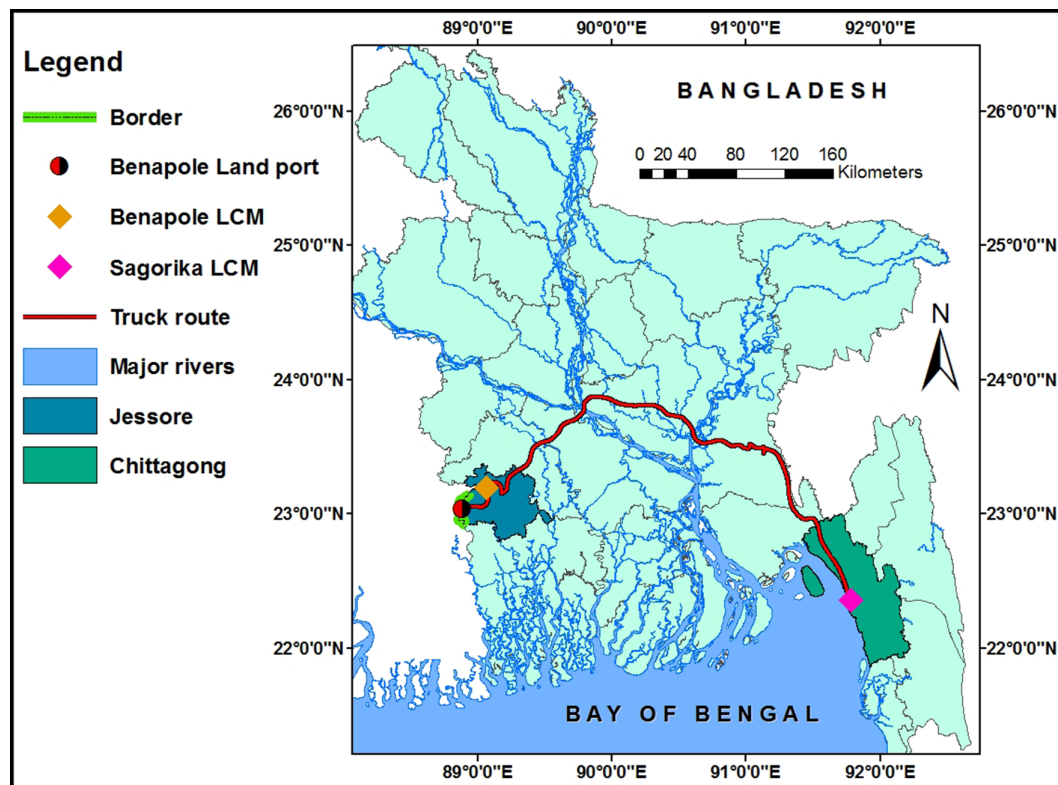


FIG 1: Map locating the selected live cattle markets of the study area. LCM, live cattle market.

in Bangladesh. Four different single-decker trucks of the same size (body size: length × width × height: 25 feet × 9 feet × 3.5 feet) and from the same vehicle manufacturer (Nitol Tata Company) were used for transporting the animals during the study period. Each trip using one single truck was made at 15-day intervals. The first author of this manuscript was accompanied by the coauthor (TMR), and they travelled by public transport each time in parallel with the selected cattle vehicle from the market of origin to the market of destination.

Transport management and environment

During transportation, the average environmental temperature and humidity were 31°C–33°C and 84–89 per cent during the day, and 26°C–29°C and 78–84 per cent during the night, respectively (Bangladesh Meteorological Department; <http://www.bmd.gov.bd>). The space allocation for each animal in the vehicle was 8–10 square feet on average per animal. Therefore, the space allocation in the vehicle restricted the animals from lying down, so they remained standing the entire transportation period. The floor of the truck was wooden and the cattle were transported without any bedding materials. The surrounding walls of the vehicles were made of iron. The transportation vehicles did not have ceilings or roof coverings; therefore the cattle were exposed to different weather conditions, such as sunlight or rain. For around half this distance, roads are often damaged with potholes, or are very uneven, which can cause sudden jerks or shaking movements of the truck during transportation. No provision of food and water was made for the animals during transportation. However, the animals were provided with paddy straw (the dried paddy plant after removal of matured seeds) and water before the start (30 minutes or 1 hour) and immediately after the end of transport. Paddy straw and water were also the sole feed for cattle for the next 24 hours when the authors collect blood samples at 24 hours after arrival in the destination market.

Selection of animals

All cattle that were proposed to be transported long distance from the market of origin (Benapole LCM) to the market of destination (Sagorika LCM) were considered to be the reference population. A total of 100 (25 from each trip) adult cattle, age two years or older, irrespective of breed and sex (mostly male), were selected for assessing health conditions (nasal discharge, diarrhoea and dehydration) and physical injuries before and after transportation. Due to limitation of funds, among the previously selected 100 cattle, blood samples were taken from 50 randomly selected cattle (13, 13, 12 and 12 from first, second, third and fourth travels, respectively) for haemato-biochemical evaluation at three time phases, viz just before the start of the trip (at the market of origin), after reaching and unloading at the market of destination, and after 24 hours of reaching the market of destination. Animal transportation usually started at

TABLE 1: Parameters for assessment of level of dehydration

Eye condition	Retention of skin fold/second	Degree of dehydration (%)
Not sunken	Absent	Mild (4–8)
Barely visible	2–4	Moderate (6–8)
Pronounced	6–10	Severe (8–10)
More pronounced	20–45	Shock (10–12)

18:00 from the source market and reached the destination market the following day at about 06:00–10:00.

Data collection

A structured questionnaire was designed in relation to the targeted objectives of the study. The information included in the questionnaire was the site and severity of injuries of animals, different health conditions such as nasal discharge, diarrhoea and dehydration, and environmental temperature and humidity (day and night). Animals were assessed closely by the first author of the manuscript twice (at the initial location and at the destination market). Animals were marked by permanent unique colour to identify the same animals at the destination site. Images of different injuries were taken with a digital camera (model no. DV 100; Samsung).

Diarrhoea was assessed by inspecting the perianal area for adhesion of faeces, observing faeces characteristics around the animal holding area, and history taken from the cattle trader on increased frequency and volume of defecation (if the trader observed and recalled this information).¹⁸ Dehydration was evaluated by looking at the condition of the eyes (sunken eye) and by performing skin fold test¹⁹ (Table 1).

Collection, transportation and preservation of blood samples

Blood samples from the selected animals were collected at the market of source (at 0 hour) and at the market of destination (immediately after arrival and then again 24 hours after arrival at the destination market).

Blood samples, 6 ml per animal, were collected from the jugular veins of the animals and placed in two separate vials: one containing EDTA for haematological evaluation and one having no EDTA for biochemical and hormonal evaluation. Each vial contained 3 ml of the blood sample. The blood samples obtained were transferred through insulated ice eskies to the laboratory at Chittagong Veterinary and Animal Sciences University between 2 and 16 hours from the destination and source market, respectively. The blood samples without anticoagulant were centrifuged at 1500 rpm for 10 minutes to separate the serum samples. The serum samples were transferred to the Eppendorf tube (2 ml) and then stored in –18°C for further analysis. The blood samples with anticoagulants were stored at 4°C. All laboratory analyses were performed within 24 hours.

Laboratory evaluation

Haematological testing

The blood samples with anticoagulant were analysed for haemoglobin (Hb), PCV, erythrocyte sedimentation rate, total leucocyte count (TLC), total erythrocyte count (TEC) and differential leucocyte count (DLC) as per the protocol described or developed by Weiss and Wardrop.²⁰

Biochemical analysis

Serum samples were evaluated for serum glucose, total protein (TP), calcium, phosphorus, alkaline phosphatase (ALP), creatine kinase (CK) and triglyceride (TG) levels using a biochemical analyser (Humalyzer 3000 Chemistry Analyzer, semiautomated benchtop chemistry photometer). The commercial kit supplied by RANDOX with its testing protocol for each selected biochemical parameter was used.

Hormonal analysis

The cortisol hormone was determined using an ELISA-based technique using the commercial kit of Monobind with its protocol (<http://www.monobind.com/site/index.html>, accessed on March 12, 2015).

Data analysis

All collected data and laboratory data were entered into Microsoft Excel 2007 and data integrity was checked. Data were then exported to the Statistical Package for the Social Sciences V.16 (SPSS V.16) software for statistical analysis. Qualitative traits like types, number and location of injuries and physical conditions were compared by McNemar's chi-square tests for paired samples. Comparisons of the quantitative parameters of serum and blood analysis between before (0 hour) and after transportation (13.8±0.9 hours and 37.8±0.9 hours) were performed using repeated-measures analysis of variance (ANOVA) followed by post-hoc tests for assessing pairwise comparison. The paired *t* test was also applied to assess the mean values of cortisol between before and after transportation. The variations of different parameters were considered significant when the P values were <0.05. The results are expressed as frequency number, percentage, mean and sd. As the floor and wall characteristics, space for animals, provision of feed and water, and other management aspects of transportation were very similar between vehicles, no confounding effect by type of transportation vehicle was therefore expected on the occurrence of outcomes. Therefore, the transport vehicle was not considered as a confounder in the statistical analysis. Transport/vehicle was not considered as a clustered variable because animals within each vehicle were not assumed as homogeneous. Therefore, no random effect of transport was expected in data analysis.

RESULTS

Approximately 13.8±0.9 (12–16) hours were required to transport animals from the market of origin (Benapole

TABLE 2: Various types of injury and their frequency distribution in cattle (before and after transportation) (n=100)

		Before (%)	After (%)	P values (McNemar's test*)
Injury types	Abrasion	11	21	0.02
	Laceration	3	8	0.06
	Swelling	2	3	1.00
	Scarification	6	6	1.00
	Barbed wire injury	9	18	0.04
	Horn fracture	2	2	1.00
Number of injury	Single	21	36	<0.001
	Double	4	8	0.13
	Multiple (>2)	1	2	1.00

*Binomial distribution used.

LCM) to the market of destination (Sagorika LCM) by road (truck). During this period enormous changes were observed in terms of animal physical health, frequency of injuries and haemato-biochemical changes.

Occurrence of physical injuries

About 26 per cent (n=100) of the cattle had injuries on different body parts that were recorded in the market of origin (Benapole LCM). However, the frequency of injuries increased significantly after long-distance transportation to the destination market (47 per cent; n=100) (Sagorika LCM) (P<0.001).

Types of injury and their frequency distribution (before and after transportation)

Six different types of injury were recorded in the studied animals. They included abrasion, laceration, swelling, scarification, barbed wire injury and bone fracture. In almost all cases the frequency of injury by type increased after transportation, where the occurrence of abrasion, laceration and barbed wire injury significantly increased after transportation (Table 2). The overall frequency of injuries increased after transportation, but single-type injury significantly increased after transportation (P<0.001) (Table 2).

Images of types of injury in different body parts of the animals are presented in Fig 2.

Frequency distribution of injury by location in cattle (before and after transportation)

Injury in different anatomical locations of animals increased after transportation, but the difference was not significant (Table 3).

Clinical assessment of animals

There was a significant difference (P=0.03) between the incidence of nasal discharge before and after transportation (15 per cent v 28 per cent). However, the occurrences of diarrhoea remained non-significant (P=0.15) between these two phases of transportation (15 per cent v 23 per

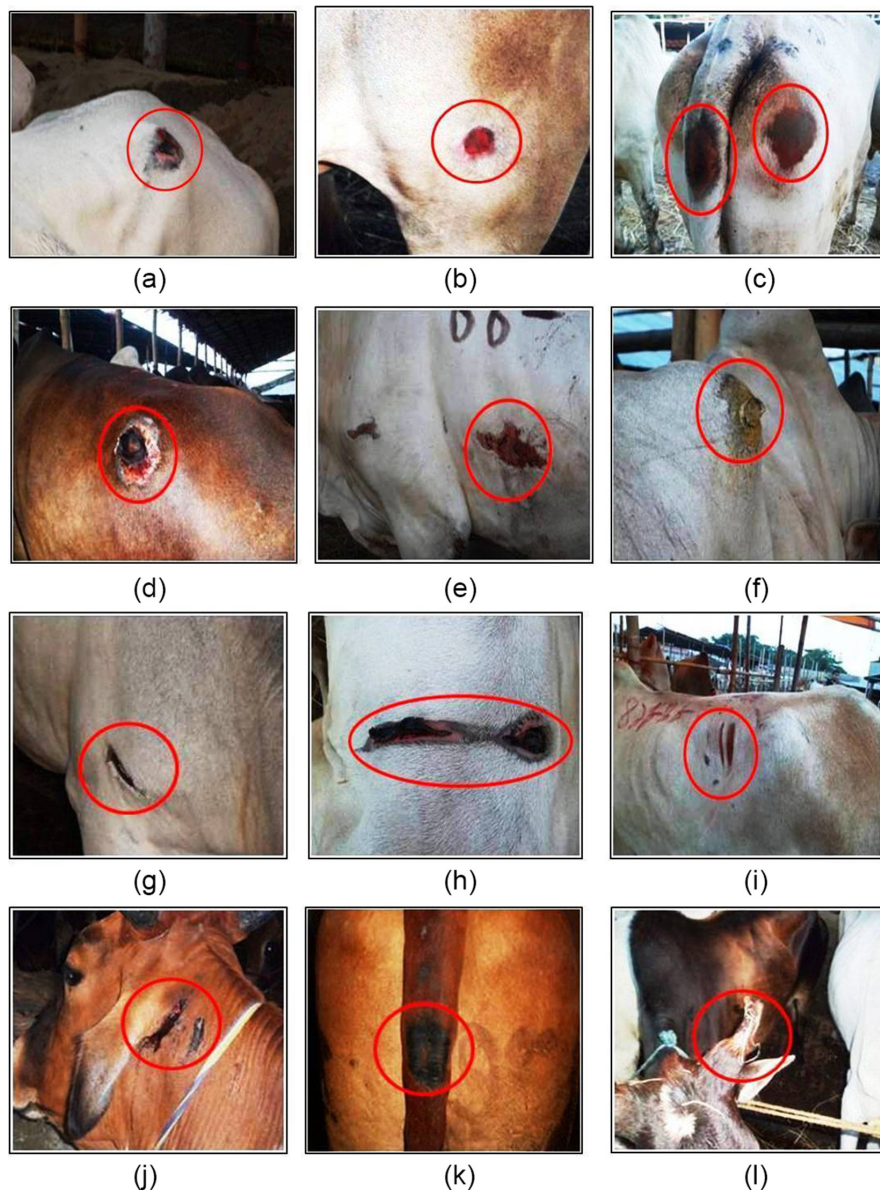


FIG 2: Different types of injuries in different anatomical locations of the animal body: (a) abrasion on the hip bone, (b) abrasion on the thigh, (c) abrasion on pin bone and tail, (d) abrasion on the paralumbar fossa, (e) abrasion on the thoracic region, (f) swelling on the back, (g) barbed wire injury on the shoulder, (h) barbed wire injury on the back, (i) barbed wire injury on the paralumbar fossa, (j) laceration on the ear, (k) scarification on the tail and (l) horn fracture.

cent). Dehydration, by category, also significantly differed before and after transportation ($P < 0.001$) (Table 4).

Haematological changes

The levels of Hb ($P = 0.01$), TEC ($P = 0.001$), TLC ($P < 0.001$), lymphocyte ($P = 0.005$), neutrophil ($P = 0.01$), eosinophil ($P = 0.01$) and the lymphocyte and neutrophil (L:N) ratio ($P = 0.006$) significantly varied before and after transportation (ANOVA). A significant increase in Hb, TEC, neutrophil and L:N ratio was observed right after transport in comparison with the pretransport values, although the values returned to pretransport levels 24 hours after the end of transportation. In the case of TLC, the value increased significantly just after transportation, where after 24 hours of transportation it decreased to significantly lower than the pretransport

value. The lymphocyte count decreased significantly just after transportation and also increased substantially at 24 hours after transportation, although the value remained lower than the pretransport one. A persistent increment of eosinophil count was observed along with the changes of phases of transportation (post-hoc test for pairwise comparison, $P \leq 0.05$) (Table 5). Among the three separate sampling times, the PCV, monocyte count and basophil count showed no variations.

Biochemical and hormonal changes

The concentration of serum TP ($P = 0.006$), ALP ($P = 0.04$), CK ($P < 0.001$) and TG ($P = 0.04$) significantly differed between before and after transportation (ANOVA). Serum TP, ALP, CK and TG were significantly higher in animals immediately after reaching the destination market than

TABLE 3: Distribution of injury by location in cattle (before and after transportation) (n=100)

Location	Percentage		P values (McNemar's test*)
	Before (%)	After (%)	
Pin bone	11	16	0.06
Back	5	7	0.50
Thigh	3	3	1.00
Ear	2	2	1.00
Horn	2	2	1.00
Thoracic region	2	4	0.50
Hip region	1	2	1.00
Paralumbur fossa	1	4	0.25
Tail	1	2	1.00
Point of hip	1	1	1.00

*Binomial distribution used.

in animals at the market of origin. However, the values of TP, ALP, CK and TG returned to the original values after 24 hours of arrival at the destination market. A significant increase in serum calcium level (ANOVA $P=0.003$) compared with the pretransport value was observed right after transport, and the value remained elevated 24 hours after the end of transportation. Non-significant variations in serum phosphorus levels were noticed between before transportation and just after transportation, although the value significantly decreased 24 hours after the end of transportation (ANOVA $P<0.001$) (post-hoc test for pairwise comparison $P<0.05$) (Table 6).

The value of serum cortisol remained unchanged before and after transportation (t test $P=0.16$) (Table 6).

DISCUSSION

The current study investigated the extent and pattern of cattle injuries, along with haemato-biochemical and hormonal changes, before and after long-distance transportation from the market of origin to the market of destination (≈ 648 km).

In the present study, the most common injuries in the cattle at the market of origin were abrasion (11 per cent), barbed wire injury (9 per cent) and scarification (6 per cent). This could be due to the cattle already

having travelled a long distance (such as from India) to reach the market of origin,²¹ as recorded during cattle trader interview at the market. During the long-distance transportation between markets, the severity of previous cattle injuries significantly increased. For example, abrasion that was 11 per cent prevalent before increased to 21 per cent after transport, and barbed wire injuries increased from 9 per cent to 18 per cent with additional transportation. This could have been a result of the overloaded trucks with an excessive number of cattle and unfair management (such as improper arrangement of cattle on vehicles, inadequate padding support, presence of pointed objects on the truck) and handling (such as quick loading and unloading, not adjusting the vehicle floor level during loading and unloading). These practices were noted and recorded in this study and have been previously supported by Minka and Ayo.²² The findings of cattle injuries in this study are also supported by a number of earlier investigators in Bangladesh.^{13 23 24} However, the cited studies had not compared cattle injuries before and after transportation. So this study was the first of its kind which quantified different cattle injuries before and after transportation.

Mismanagement during animal transport seriously impacts animal welfare by exerting significant stress to the body.²¹ The present study recorded animal deprivation from feed and water, insufficient space allocation (average 8–10 square feet per animal) for the animals in the vehicles, and forcefully keeping the animals in standing position for the entirety of transportation. The deprivation of feed and water is considered a violation of freedom from hunger, which is one of the five needs used in assessing animal welfare.²⁵ Fasting and long standing times may have an effect on low serum glucose concentration (also discussed later in this manuscript) and malfunctioning of the body.²⁶ The space per cattle in the vehicle during transportation in this study was estimated to be 8–10 square feet per animal, which is lower than the standard space requirement for Zebu cattle (*B indicus*) transportation via road vehicles. According to the Bureau of Indian Standard, about 15–20 square feet per cattle is required.²⁷ The high stocking density of cattle in the vehicle during transportation may have contributed significantly to the increase in the frequency of severe injuries during transportation.

The feeding of low-quality forages was also documented in the market of destination in the current study where the cattle were fed only with paddy straw and drinking water. This deprivation from nutritional needs also hinders animal welfare.²¹

The presence of nasal discharge increased by 13 per cent in the cattle after transportation (15 per cent before to 28 per cent after) at the destination market in the current study. This finding is in close agreement with the finding of Ishizaki and others⁷ and Mitchell and others.⁹ High frequencies of nasal discharge in cattle after transportation might have resulted from invasion of microorganisms into the upper respiratory tract^{28–30} due

TABLE 4: Level of dehydration among the cattle before and after transportation (n=100)

Variable	Categories	Percentage		P values (McNemar's test*)
		Before	After	
Dehydration	Mild	49	15	<0.001
	Moderate	43	65	<0.001
	Severe	8	20	<0.001

*Binomial distribution was used.

**TABLE 5:** Comparison of mean values of haematological parameters before and after transportation of animals (n=50)

Parameters	Before transportation	After transportation		P values (ANOVA)
	0 hour (at origin) Mean±sd	13.8±0.9 hours (at destination) Mean±sd	37.8±0.9 hours (24 hours after arrival at destination) Mean±sd	
Haemoglobin (mg/dl)	11.1±2.6 ^a	12.3±3.2 ^b	10.6±2.9 ^a	0.01
Packed cell volume (%)	30.1±5.2	32.4±5.4	30.3±5.2	0.08
Total erythrocyte count (10 ⁶ /µl)	4.7±1.6 ^a	5.7±1.2 ^b	4.5±1.0 ^a	0.001
Total leucocyte count (10 ³ /µl)	6.2±1.5 ^b	7.3±1.3 ^c	5.3±0.3 ^a	<0.001
Lymphocyte (%)	61.7±4.5 ^b	58.1±3.8 ^a	60.6±4.8 ^b	0.005
Monocyte (%)	4.8±1.6	4.3±2.4	4.2±1.8	0.18
Neutrophil (%)	29.7±3.9 ^a	32.8±4.5 ^b	30.2±4.6 ^a	0.01
Eosinophil (%)	3.8±1.6 ^a	4.7±2.6 ^b	5.3±2.3 ^b	0.01
Basophil (%)	0.1±0.3	0.1±0.3	0.1±0.3	1.00
L:N	0.48±0.1 ^a	0.56±0.1 ^b	0.50±0.1 ^a	0.006

Means with different superscripts in the same row differ significantly (P<0.05). ANOVA, analysis of variance; L:N, lymphocyte and neutrophil ratio.

to immune suppression by transportation stress,⁶ invasion of dust³¹ and heat stress.³²

The current study noticed an 8 per cent increase in the frequency of diarrhoea in the cattle after transportation from 15 per cent before to 23 per cent after. This finding is partially in line with the outcome of the previous study conducted by Richeson and others,³³ where they transported 264 calves from western Arkansas and eastern Oklahoma to the University of Arkansas Agricultural Experiment Station, USA. Increase in the frequency of diarrhoea may be due to proliferation and intestinal invasion of pathogenic organisms (such as *Escherichia coli*) in stressed, immunocompromised animals.³⁴

The frequency of severe dehydration in the cattle also increased by 12 per cent in the present study, from 8 per cent before to 20 per cent after, which converges

with reports published by Villarroel and others³⁵ and Hogan and others.³⁶ The increased occurrence of severe dehydration could be a result of water deprivation, high evaporative water loss due to high ambient temperatures and water loss through diarrhoea.^{37 38}

The elevations of Hb by 9.8 per cent (11.1 mg/dl before and 12.3 mg/dl after) and TEC by 17.5 per cent (4.7×10⁶/µl to 5.7×10⁶/µl) in the cattle after transportation in the present study indicate haemoconcentration, which could be due to the effect of diarrhoea and dehydration during transportation. These findings and the given logic have coincided with the findings of earlier studies.^{39 40} They also found an increase of Hb and TEC in cattle, respectively, by 9.9 per cent and 14.3 per cent after 27 and 18 hours of transportation. In contrast, various studies performed previously reported the unchanged Hb and TEC values in cattle after

TABLE 6: Comparison of mean values of biochemical parameters before and after transportation of animals (n=50)

Parameters	Before transportation	After transportation		P values (ANOVA)
	0 hour (at origin) Mean±sd	13.8±0.9 hours (at destination) Mean±sd	37.8±0.9 hours (24 hours after arrival at destination) Mean±sd	P values (t test)*
Glucose (mg/dl)	40.6±17.0	36.0±18.0	36.8±16.5	0.30
Total protein (g/dl)	6.8±1.3 ^a	8.2±1.9 ^b	6.9±1.4 ^a	0.006
Calcium (mg/dl)	11.3±2.2 ^a	13±2.7 ^b	12.4±1.0 ^b	0.003
Phosphorus (mg/dl)	7.3±1.3 ^b	7.6±2.5 ^b	5.3±2.2 ^a	<0.001
Alkaline phosphatase (U/l)	330.0±89.5 ^a	363.0±123.8 ^b	327.0±82.5 ^a	0.04
Creatine kinase (U/l)	574.9±33.4 ^b	1288.0±345.4 ^c	469.1±146.9 ^a	<0.001
Triglyceride (mg/dl)	104.7±33.4 ^a	127.7±37.2 ^b	116.3±47.5 ^a	0.04
Cortisol µg/dl*	4.0±2.6	4.8±2.6	–	0.16

Means with different superscripts in the same row differ significantly (P<0.05).

*Only measured two times: before and just after transport.

ANOVA, analysis of variance.

transportation, which could be due to a short-distance transportation (<10 hours) and the provision of enough water and space during transportation.⁴⁹

The level of TLC increased just after transportation (6.2 thousands/ μ l before to 7.3 thousands/ μ l after) and then decreased to reach 5.3 thousands/ μ l in this study, which corresponds to other studies elsewhere in Bangladesh⁴¹ and neighbouring countries.⁷ The pattern of TLC changes might be due to the stimulating effect of glucocorticoids on TLC during long-distance transportation.⁴²

The level of lymphocytes decreased (61.7 per cent before to 58.1 per cent after) just after transportation and then again returned to the original value of 60.6 per cent (24 hours after the completion of transportation), whereas the level of neutrophils significantly increased (29.7–32.8 per cent) just after transportation. These patterns of lymphocyte and neutrophil changes align with the findings of many other studies performed elsewhere in the world.^{6,9,37,43} The reduction of lymphocytes in the current study may be explained by the fact that lymphocytes usually receive stimulations by glucocorticoid and adrenergic receptors,⁴⁴ on their surfaces, but in response to stress these receptors are downregulated.⁴⁵ The increment of neutrophil count in this study may be a proportionate increase that may be a result of lymphopenia, which is quite common in stressed animals.⁴⁶ The possible cause of normalised lymphocyte count after 24 hours of transportation is the reactivation of adrenergic receptor due to elapsed time.⁴⁷ Contrarily, various studies reported unchanged DLCs in cattle before and after transportation,^{9,48} which could be due to short-distance transportation in conjunction with maintaining a good transportation management system.

The ratio between neutrophils and lymphocytes was significantly increased after transportation (0.5:1 before and 0.6:1 after) in the present study, which is similar to the findings reported by Stockman and others.⁴³

No significant variation of serum glucose concentration was recorded in the cattle before (40.6 mg/dl) and after transportation (36.0 mg/dl) in the present study, which aligns with a previous study.⁴³ The relatively unchanged serum glucose concentration may be due to the mobilisation of body energy reserve (TG)³⁷ by the activity of adrenaline and noradrenaline, the two hormones that are secreted during transportation stress.⁴⁹ However, a significant reduction of serum glucose concentration in the cattle was reported after transportation (43.8 mg/dl before to 31.9 mg/dl after).^{4,48} This decline of glucose concentration could be due to psychological stress resulting from a novel environment and unfamiliar animals and handlers.⁵⁰ Some earlier studies estimated a significant increase of serum glucose concentration in cattle after 24-hour transportation (100.6 mg/dl before to 106.6 mg/dl after).³⁷

The present study found significantly increased TP, CK and TG concentrations in the cattle just after transportation (6.8 g/dl before to 8.2 g/dl after, 574 U/l before to 1288 U/l after and 104.7 mg/dl before to

127.7 mg/dl after, respectively) and then regressed to the original value (6.9 g/dl, 469.1 U/l and 116.3 mg/dl, respectively). These findings are similar to a number of earlier studies.^{37,39,51,52} The referred studies determined 7.2–7.9 mg/dl TP, 1220–1800 U/l CK and 105–120 mg/dl TG after transportation. The estimated level of these biochemical parameters could have occurred due to different mechanisms: (1) promotion of protein metabolism by increased level T_3 secretion during transportation, which might be responsible for the increased level of TP—the post-transport dehydration leading to loss of water from the blood and therefore haemoconcentration may also be responsible for the increased level of TP⁵³; (2) muscle breakdown due to the long-time muscle activity during transportation, which could be responsible for the elevation in serum CK concentration³; and (3) mobilisation of stored TGs with the support of adrenaline and noradrenaline hormones secreted during transportation could be the reason for the increase in serum TG concentration.^{37,54} However, there are several studies that are inconsistent with the current findings of these biochemical parameters.^{51,55,56} They found decreased concentration of these biochemical parameters after transportation (TP: 5.7 mg/dl before to 5.1 mg/dl after; CK: 200 U/l before to 110 U/l after; and TG: 58.1 mg/dl before to 52.3 mg/dl after). The reason behind the lower level of these biochemical parameters compared with the current study might be due to a short-distance transportation (\leq 8 hours).⁵⁷

In the current study, the serum ALP concentration increased just after transportation (330 U/l before to 363 U/l after) and then returned back to the original value, which is an analogous result to the report of a previous study in beagle dogs.⁵⁸

Increased serum calcium (11.3 mg/dl before to 13 mg/dl after) and phosphorus (7.3 mg/dl before to 7.6 mg/dl after) concentrations were reported in the present study, which might be due to hyperparathyroidism activity in maintaining blood calcium concentration in long-distance transportation.⁵⁹

The concentrations of cortisol (endocrine hormone) insignificantly increased after transportation (4 μ g/dl before to 4.8 μ g/dl after) in the present study. The similar results are reported by a number of previous studies.^{4,51} This particular trend could be explained by the variable duration of transportation time. The level of cortisol increases to peak at 1.5–4 hours of transportation and then gradually decreases with the increase of transportation time due to the negative feedback to the hypothalamus by the initial peak concentration of cortisol in serum.^{60,61}

However, many earlier studies found cortisol elevation at a significant level after transportation, such as 10 ng/ml (before) to 22 ng/ml (after),⁵⁵ and 4.8 ng/ml (before) to 15 ng/ml (after).⁶² The mechanism behind the increase of blood cortisol level can be explained as signals, originating from stress such as long-distance transportation, are transmitted to the hypothalamus, activating HPA and sympathetic-adrenal axes. The HPA axis affects perception in the brain,

resulting in the release of the hypothalamic factor, corticotrophin-releasing factor and vasopressin, which in turn stimulates the anterior pituitary gland to secrete adrenocorticotropic hormone (ACTH). The ACTH circulating in the blood stimulates the adrenal cortex to surge of cortisol secretion.^{5 63 64}

Limitations of the current study

Selection bias might be introduced as the sample size for the study was not calculated based on statistical assumptions and random sampling was not always followed.

Information and interview biases might be introduced because traders sometimes appeared worried about legal action if they provided accurate information. However, it was ensured that the authors maintained the confidentiality of the information. As information on the breed and sex of the animals was not recorded, a limitation is that the authors do not know if and how these factors influenced the findings of the study.

Measurements of space allocation per animal in the vehicle might not have been as precise as the authors would have liked. The diagnostic error might have occurred as the diagnostic tests that the authors used were not 100 per cent sensitive and 100 per cent specific.

CONCLUSION

The transportation system for animals in Bangladesh is marked by a lack of oversight or regulation, where animals can be subject to cruelty and rough handling. The frequency of the different types of injuries such as abrasion, barbed wire injuries, scarification, laceration and so on with the degree of severity in the cattle increased after transportation. The high stocking density of the cattle made to stand in the vehicle, in addition to feed and water deprivation, was recorded during transportation. Only paddy straw and a small amount of drinking water were offered to the animals at the destination market. The high frequency and severity of injuries, poor health conditions and enormous haemato-biochemical changes during transportation clearly reflect the poor welfare of the cattle during transportation in Bangladesh.

Cattle stocking density limits in vehicles, ventilation facilities, and provision of quality feed and water should be ensured during long-distance transportation to prevent the incidence of different types of cattle injuries, respiratory problem, diarrhoea, dehydration, immune-suppression, and detrimental haemato-biochemical and hormonal changes with the aim of preserving animal welfare. Necessary government rules and regulations should be developed and implemented in practice, along with conducting traders' education and incentive programmes to ensure animal welfare in Bangladesh.

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