



**The consequences  
of different  
external factors  
on animal welfare  
during long-distance  
transport**

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## DEFINITIONS

**BEDDING** – is considered non-compliant when it does not meet the following requirements: adequate bedding material should be dry and able to absorb liquids well and the floor surface is not visible. Sufficient amounts of bedding allow for more comfort and facilitate the resting of animals. If bedding is dirty and wet with faeces and urine, it is considered inadequate (if >25% of the compartment floor is dirty).

**CORRECT SEPARATION OF ANIMALS** – considered non-compliant according to Regulation 1/2005 if animals of different sizes/ages/shorn/unshorn are mixed. This means the animals of significantly varied sizes or ages, animals with horns being transported together with animals without horns and animals displaying hostility to one another.

**DOWNER** – considered if the animal is unable to stand on its own.

**DISTANCE TO THE PLACE OF THE INSPECTION** – the distance between the place of departure and the place where the truck was inspected (i.e. Bulgarian-Turkish border).

**DRINKING SYSTEM** – considered depending on whether the drinking system was available and usable for transported animals during inspection or not (NOT IN USE means: unsuitable for the type of animal, broken, turned off (while the truck was waiting for hours in the sun without a shade) or dirty (contaminated with faeces, straw or other materials and therefore unfit for consumption). On the other hand, water supply was considered adequate when cattle received water manually by the drivers using extra water buckets.

**FATIGUE** – an extremely tired/exhausted animal due to muscles unable to work at the same level of intensity any longer.

**HEADSPACE** – adequate space above the animals so that they may adopt a natural standing position and there is no hindrance to natural movement, allowing optimum ventilation.<sup>1</sup> It is considered not adequate when parts of the animal's back touch the ceiling or sufficient the internal height is not guaranteed, which for cattle is interpreted as at least 20 cm above the height at the withers of the tallest animal<sup>2</sup>

**HEAT STRESS** – when animals are responding to the excessive heat load.

**INSIDE RELATIVE HUMIDITY** – was measured with BOSCH PD1® thermo detector ONLY at one stage of the journey at the border (on the Bulgarian or Turkish side, or both). **NOTE that this parameter was not measured during the whole journey.**

**INSIDE THI** – Temperature Humidity Index. The parameter was calculated using the following formula:  $INSIDE\ THI = 0,8 * TEMP + RH * (TEMP - 14,4) + 46,4$ .

**LOADING DENSITY** – if animals cannot turn around and do not have enough space to lay down in a natural position, the space allowance is considered inadequate. The trucks are considered non-compliant if the following requirements are not met:

- The animals should have sufficient space so that they can turn around easily.<sup>1</sup>

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<sup>1</sup> Consortium of the Animal Transport Guides Project (2017). "Guide to good practices for the transport of cattle".

<sup>2</sup> SANCO G3 AN/ap D(2011) 86223 based on "Scientific Opinion Concerning the Welfare of Animals during Transport<sup>3</sup>" adopted by the European Food Safety Authority (EFSA) on 2 December 2010 and published on 12 January 2011.

- Unshorn sheep and lambs of  $\geq 26$  kg with thick fleece should be offered 25% more space than shorn sheep<sup>1</sup>.

MORTALITY – defined as the “uncontrolled” death of an animal as well as cases of euthanasia and emergency slaughter during the transport (**NOTE that dead animals after long-distance transport were not measured**).

NASAL DISCHARGE – when mucus runs from the nose. Also called post-nasal drip or rhinorrhoea.

ORIGIN COUNTRY DEPARTURE – the country that approved the journey.

PANTING – controlled increase in respiratory frequency accompanied by a decrease in tidal volume. It is SCORED according to these tables separately for cattle and sheep: <https://bit.ly/3wXWEmF> .

ROLLING TONGUE – an animal’s tongue is outside and rolled back inside the mouth.

SWEATING (IN CATTLE) – the release of liquid from the animal’s sweat glands. Sweating is a major way cattle use to control their body temperature and avoid overheating.

TEMPERATURE INSIDE VEHICLE – was measured with BOSCH PD1P® thermo detector ONLY at one stage of the journey at the border (on the Bulgarian or Turkish side, or both) and is considered non-compliant when it does not meet the following requirements: the temperature is below 0 and above 35°C. According to Regulation 1/2005, the acceptable temperature range is between 5 to 30°C, with a tolerance of 5°C. For sheep consignments, the temperature was provided in Fahrenheit. **NOTE that this parameter was not measured during the whole journey.**

TIME FOR BORDER CROSSING – time that truck needs to fully cross the Bulgarian and Turkish borders. Special characteristics of these border crossings are different working hours; 24/7 on the Bulgarian side and from 9 am to 5 pm, plus bureaucracy, on the Turkish side. These factors, as well as others that are not mentioned, lead to long line-ups and waiting times. If crossing both takes more than two hours, it is considered non-compliant.

VENTILATION – it is considered whether the ventilation system is available and usable for transported animals during inspection or not (NOT IN USE means: not existing, broken or turned off (while the truck is waiting for hours without a shade).

UNFIT – animals are considered unfit for transport when they:

- have fallen or been trampled on or are injured. e.g., as a result of aggression, and have a clear lesion or fracture;
- exhibit an injury, such as a hernia or a prolapse or a dislocation;
- exhibit symptoms of heat or cold stress and/or dehydration;
- appear to have developed symptoms of a disease or infection;
- give birth during the journey;
- are downer animals.

## 1. OBJECTIVE

This study aims at providing statistical information on when a given **External Factor (EF)** is correlated with an **Animal-Based Indicator (ABI)**.

## 2. BACKGROUND

The analysed data were collected by Animal Welfare Foundation during an inspection carried out on the Bulgarian and Turkish border (Kapitan Andreevo – Kapikule) between 2016 and 2021.

In this summary, only the results of the truck inspections where ABIs were observed and recorded were used for the final analysis. Inspection times varied from truck to truck, with times affected by the availability of the drivers to let our teams inspect their trucks. It is important to add that only compartments of the first decks of the trucks were inspected, as our teams could not inspect the upper decks due to time limit, non-collaboration of the drivers and safety reasons. The inspections were carried out on the Bulgarian (Kapitan Adreevo) or Turkish (Kapikule) side of the border, or both. No inspections were done in the final stage of the journey.

## 3. METHODOLOGICAL CONSIDERATIONS

This study was performed with 112 consignments with cattle. To obtain meaningful results on the correlation between EFs and ABIs, adequate statistical tests must be selected.

In this study, there are two types of variables:

**Categorical variables:** Qualitative, without numbers but with text. In some cases, there is an order that should be considered (e.g. for the temperature inside the vehicle, *not exceeding 30 < exceeding 30 < exceeding 40*)

**Numerical variables:** Quantitative (e.g. mortality)

### 3.1 CATEGORICAL VARIABLES

**External Factors (EFs):**

- Animal category (general)
- Origin country departure
- Heat stress scale (Bulgaria (BG))
- Heat stress scale (Turkey (TR))
- Heat stress scale TOTAL (BG-TR)
- Temperature inside vehicle TOTAL (BG-TR)
- Bedding TOTAL (BG-TR)
- Headspace TOTAL (BG-TR)

- Loading density TOTAL (BG-TR)
- Correct separation of animals (mixed sizes) TOTAL (BG-TR)
- Correct separation of animals (mixed horns and hornless) TOTAL (BG-TR)
- Drinking system TOTAL (BG-TR)
- Ventilation TOTAL (BG-TR)
- Sheep: shorn/unshorn TOTAL (BG-TR)

#### **Animal-Based Indicator (ABIs):**

- Mortality TOTAL (TR-BG)
- Fatigue TOTAL (TR-BG)
- Downer/unfit TOTAL (TR-BG)
- Fighting for water TOTAL (BG-TR)
- Drinking from urine/eating filthy bedding TOTAL (BG-TR)
- Biting/licking bars/nipples TOTAL (BG-TR)
- Rolling tongue TOTAL (BG-TR)
- Nasal discharge TOTAL (BG-TR)
- Cattle: sweating TOTAL (BG-TR)

### 3.2. NUMERICAL VARIABLES

#### **External Factors (EFs):**

- Distance to destination
- Time for border crossing
- Inside temperature (BG)
- Inside relative humidity (BG)
- Inside THI (BG)
- Inside temperature (TR)
- Inside relative humidity (TR)
- Inside THI (TR)

#### **Animal-Based indicators (ABIs):**

- Cattle: panting score TOTAL (BG-TR)
- Sheep: panting score TOTAL (BG-TR)

When the objective is to study the correlation of two categorical variables, where a specific order is not found, we apply:

- (I) Fisher's exact test when we have less than 5 different observations in each variable type (e.g., trucks from any country > 5);
- (II) Pearson's  $\chi^2$  of independence where there are more than 5 observations.

When the objective is to study the correlation of a categorical variable (mainly ABIs) with numerical variables (EFs such as temperature, humidity, etc.), we apply:

- (I) the t-test when numerical variables are normally distributed;
- (II) the Wilcoxon test when numerical variables are not normally distributed.

The Shapiro-Wilk test is used as a test of normality.

When the objective is to study the correlation of two numerical variables (e.g., panting score and temperature), a model of analysis of variance was applied, using Tukey Honest Significant Differences, and the variables were evaluated individually.

Not assigned (NA) values have not been included to perform the test correctly. The null hypothesis is that both variables are not correlated, while the alternative hypothesis is that they are correlated. The smaller the p-value, the stronger the evidence that the null hypothesis should be rejected. A p-value less than 0,05 (typically  $\leq 0,05$ ) is statistically significant. It indicates strong evidence against the null hypothesis, as there is less than a 5% probability the null is correct (and the results are random). A p-value  $\geq 0,05 \leq 0.1$  indicates a trend against the null hypothesis, with less than a 10% probability that the null hypothesis is correct.

#### **Partial Least Squares (PLS)/Figure 4**

PLS creates components (understood as linear combinations of the different EF results) to explain the maximum variance of the data. Therefore, the model is created to evaluate the correlation between the EFs and the ABIs observations within one analysis. In the biplot of the PLS (Figure 4), those EFs (marked as blue dots) that correlate with specific ABIs (marked as green dots) appear in the same area of the plot. Finally, each orange hexagon corresponds to a single consignment. In the figure, the X-axis of the plot indicates *non-appropriate* ABI values (e.g., the presence of dead animals). The Y-axis groups EFs that are correlated with each other but with limited (or no) effect on the ABIs.

Therefore, EFs located on the right side of the X-axis and in the centre of the Y-axis are correlated with those *non-appropriate* ABI values. It includes *distance to the place of the inspection, headspace, bedding total, time for border crossing* and *loading density*. Those EFs located to the right of the plot, but in the upper part of the Y-axis (rounded by a black circle) are correlated between them, but do not show important effects on ABIs. Those values are *THI, heat stress, temperature and humidity inside the vehicle*. Those results are consistent with the individual analysis (Table 1, Figure 1, Figure A1), where the correlation of these observations with ABIs is statistically significant individually.

**Distance to the place of the inspection clarification:** At first glance, the results seemed to indicate that a larger *distance to the place of the inspection* resulted in better ABIs (some positive correlations were found). The parameter *distance to the place of the inspection* is strongly linked with the *country of origin* (which shows a clear correlation with several ABIs). There are countries of origin with better and higher standards of journey approval. Since some of the countries with better standards (e.g., the Netherlands or Germany) travel longer distances than other countries with lower standards, this type of “wrong” positive association occurred. Therefore, correlations found for *distance to the place of the inspection* have been discarded.

## 4. SUMMARY OF THE RESULTS AND MAIN CONCLUSIONS

### 4.1 CATTLE

The evaluation of the data showed statistically significant correlations between several of the evaluated EFs and ABIs in cattle. Specifically, **eleven of the fourteen evaluated EFs showed at least one significant correlation.**

**Table 1** presents a comprehensive summary of the ABIs that showed a significant statistical correlation with each evaluated EF in cattle. Similarly, **Figure 1** shows a simplified heatmap of those correlations. Red points represent statistically significant correlations (ranging from intense red to light red (from  $p$ -value 0 to 0.1) between ABIs and EFs, while green points represent no significant correlation. The same table with the specific  $p$ -value for each analysis is available in the annexe (**Table A1**) at the end of the document.

The parameter *bedding* was the one with a significant statistical effect on the highest number of ABIs. When this parameter was considered non-compliant, it showed an effect on seven ABIs including *mortality*, *downer*, *unfit*, *fighting for water*, *drinking from urine/eating filthy*, *biting/licking bars/nipples*, *rolling tongue*, *sweating* and *panting score value*. A representative example of the effect on these parameters can be observed in how adequate/inadequate *bedding* affected the *panting score* (**Figure 2**). When this parameter was considered adequate, 70% of the consignments showed no panting (panting score 0), 24% had a panting score of 1 and only 6% of the consignments showed a panting score of 2. However, when this parameter was considered inadequate, the percentage of consignments with no panting was reduced to 50%, and 25% showed a panting score  $\geq 2$ , reaching scores  $\geq 4$  in 15% of the cases. Another relevant EF was the *loading density*, with a direct effect on the *mortality*, *fatigue*, *downer*, *unfit*, *fighting for water* and *biting/licking bars*. When this parameter was inadequate, the presence of dead animals was found in 11% of the consignments, while this percentage was reduced to 0% when the *loading density* was adequate (**Figure 3**).

Additional EFs that showed significant correlations with the presence of *dead animals* in the consignments include *time for border crossing*, *headspace*, *country of origin* and *drinking system*.

Those correlations were also observed when applying multivariate analysis. The discriminant analysis PLS was applied to observe whether some of these EFs are correlated at the same time with ABIs (**Figure 4**). The results confirm the conclusions showing that EFs correlated with a larger amount of ABIs are located in the same zone of the biplot. A more detailed explanation of Figure 4 is available in the methodology considerations section.

Besides, other parameters showed much more limited effects. The *temperature inside the vehicle*, *heat stress* (strongly related to the first one) and *correct separation of animals by horns* did not show any statistically significant correlation with ABIs, while the correct separation of animals by size and the THI only showed a correlation with *fatigue* (and with *downer* in the case of trucks inspected in Bulgaria).

**Table 1:** Summary of the ABIs that showed a significant statistical correlation with each evaluated EF.

External Factor (EF)	Number of ABIs affected*	Animal-Based Indicators (ABIs)
Bedding (BG+TR)	7+0	<ul style="list-style-type: none"> <li>➤ Mortality</li> <li>➤ Downer unfit,</li> <li>➤ Drinking from urine/eating filthy</li> <li>➤ Biting/licking bars</li> <li>➤ Rolling tongue</li> <li>➤ Sweating</li> <li>➤ Panting score</li> </ul>
Loading density (BG+TR)	4+1*	<ul style="list-style-type: none"> <li>➤ Mortality</li> <li>➤ Fatigue total</li> <li>➤ Fighting for water</li> <li>➤ Biting/licking bars</li> <li>➤ Downer unfit*</li> </ul>
Inside THI (BG+TR)	3+0	<ul style="list-style-type: none"> <li>➤ Fighting for water</li> <li>➤ Rolling tongue</li> <li>➤ Nasal discharge</li> </ul>
Headspace (BG+TR)	2+1*	<ul style="list-style-type: none"> <li>➤ Mortality</li> <li>➤ Panting Score</li> <li>➤ Biting/licking bars*</li> </ul>
Origin country	2+1*	<ul style="list-style-type: none"> <li>➤ Fighting for water</li> <li>➤ Sweating</li> <li>➤ Mortality*</li> </ul>
Distance to a place of inspection	0+0 <sup>&amp;</sup>	-
Time for border crossing	2+0	<ul style="list-style-type: none"> <li>➤ Dead total</li> <li>➤ Sweating</li> </ul>
Correct separation mixed sizes total (BG+TR)	1+0	<ul style="list-style-type: none"> <li>➤ Fatigue total</li> </ul>
Humidity inside the vehicle (BG+TR)	1+0	<ul style="list-style-type: none"> <li>➤ Rolling tongue</li> </ul>
Drinking system total (BG+TR)	0+2*	<ul style="list-style-type: none"> <li>➤ Mortality*</li> <li>➤ Drinking from urine/eating filthy*</li> </ul>
Ventilation total (BG+TR)	0+0	-
Temperature inside vehicle total (BG+TR)	0+0	-
Heat stress scale (TR+BG)	0+0	-
Correct separation mixed horn/hornless(BG+TR)	0+0	-

\*p-value < 0.1 ≥ 0.05

<sup>&</sup>See “methodological considerations/distance to destination clarification”

EF \ ABI	Mortality	Fatigue	Downer unfit	Fighting for water	Drinking Urine / Eating filthy	Biting/ Licking bars	Rolling tongue	Nasal Discharge	Sweating	Panting Score
Bedding total (BG+TR)	Red	Green	Light Red	Light Green	Red	Red	Red	Light Green	Red	Red
Loading density total (BG+TR)	Red	Red	Light Red	Red	Green	Red	Light Green	Green	Light Green	Light Green
Inside THI TOTAL (BG+TR)	Light Green	Light Green	Light Green	Red	Light Green	Light Green	Light Red	Red	Green	Light Green
Origin country	Light Red	Green	Light Green	Red	Light Green	Light Green	Light Green	Light Green	Red	Light Green
Time for border crossing	Red	Light Green	Green	Light Green	Light Green	Green	Light Green	Light Green	Red	Light Green
Headspace total (BG+TR)	Red	Green	Green	Green	Green	Light Red	Light Green	Light Green	Light Green	Light Red
Humidity inside vehicle TOTAL (BG+TR)	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Red	Light Green	Light Green	Light Green
Correct separation mixed sizes total (BG+TR)	Light Green	Light Red	Green	Light Green	Light Green	Light Green	Green	Green	Light Green	Light Green
Temperature inside vehicle TOTAL (BG+TR)	Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green
Heat stress scale (TR+BG)	Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green
Correct separation mixed horn/hornless(BG+TR)	Green	Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green	Light Green
Drinking system total (BG+TR)	Light Red	Light Green	Green	Light Green	Light Red	Light Green	Light Green	Light Green	Light Green	Light Green
Ventilation total (BG+TR)	Green	Light Green	Light Green	Light Green	Light Green	Light Red	Light Green	Light Green	Light Green	Light Green

**P-value<0.05**

**P-value<0.10**

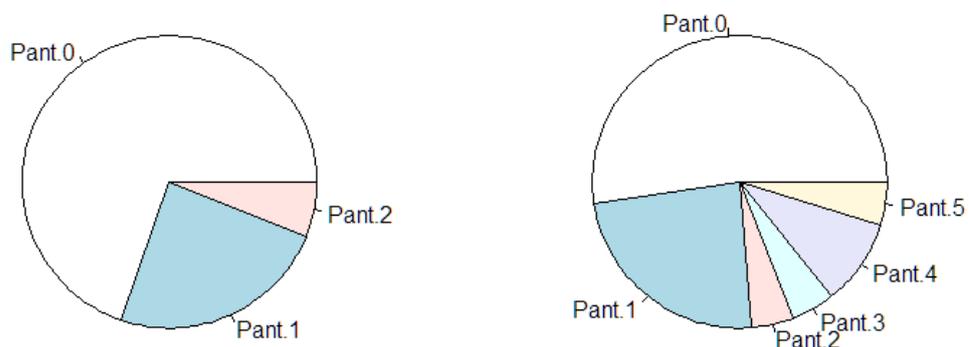
**P-value>0.10**

**P-value 1**

**Figure 1:** Simplified heat map of the correlations between ABIs and EFs.

### Adequate bedding

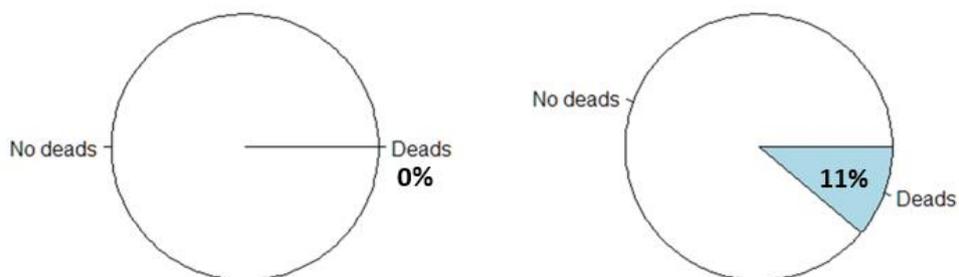
### Inadequate bedding



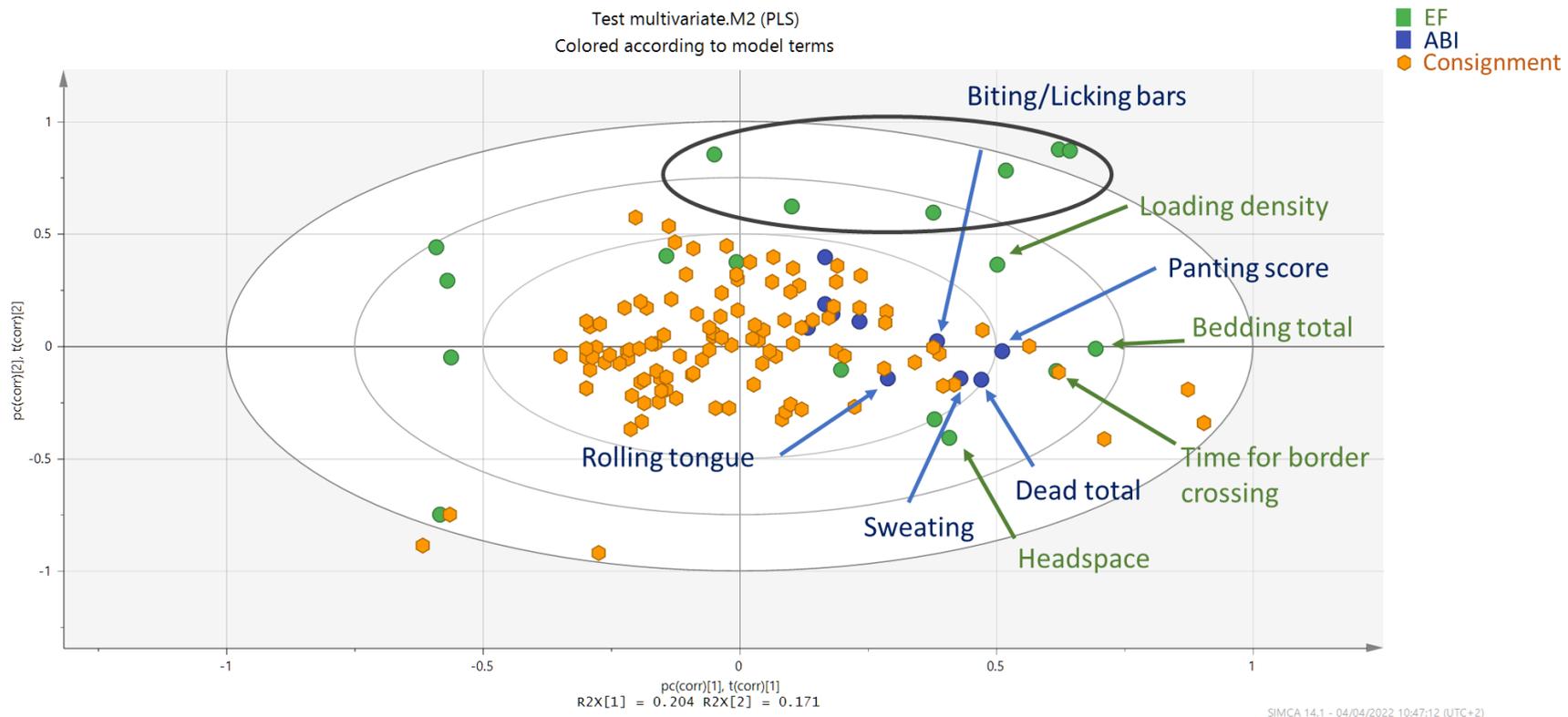
**Figure 2:** Effect of adequate/inadequate bedding on the panting score of cattle.

### Adequate Loading density

### Inadequate Loading density



**Figure 3:** Effect of the loading density on the presence of dead animals.



**Figure 4:** Biplot of the Partial Least Squares analysis.

## 4.2 SHEEP

Only six consignments with sheep were inspected, i.e. the available data are insufficient to perform meaningful and correct statistical analysis. However, it does not imply that sheep consignments are transported properly.

**Table 2** summarizes the results of the evaluated ABIs. Six out of the ten ABIs studied showed at least one inappropriate result (understood as *bad* (e.g., presence of dead animals, animals with fatigue, animals with nasal discharge, etc)). It is noteworthy that all the consignments carried animals with fatigue and dead animals were reported in five of the six trucks inspected. These preliminary results suggest that sheep are incorrectly transported, but more consignments must be studied to obtain conclusive results about which EFs are affecting them.

**Table 2:** Summary of the observed ABIs in the six evaluated consignments.

	Appropriate	Inappropriate
<b>Dead TOTAL (TR-BG)</b>	5	1
<b>Fatigue TOTAL (TR-BG)</b>	0	6
<b>Downer/unfit TOTAL (TR-BG)</b>	5	1
<b>Fighting for water TOTAL (BG-TR)</b>	6	0
<b>Drinking from urine/eating filthy, bedding TOTAL (BG-TR)</b>	6	0
<b>Biting/licking bars/nipples TOTAL (BG-TR)</b>	5	1
<b>Nasal discharge TOTAL (BG-TR)</b>	5	1
<b>Panting score TOTAL (BG-TR)</b>	1(1)	2(5)

## VARIABLES

In the following tables and graphs, we present detailed information for each EF concerning each ABI.

**Table 3: MORTALITY**

First variable (categorical)	Second variable	Observation type (2 <sup>nd</sup> variable)	Number of observations in each group	Statistic test	p-value
<b>Mortality</b>	<b>Origin country</b>	<b>Categorical</b>	<b>3/91</b>	<b>Fisher</b>	<b>0.098</b>
<b>Mortality</b>	<b>Distance to the place of inspection</b>	<b>Numerical (No normal)</b>	<b>3/52</b>	<b>Wilcoxon test</b>	<b>0.015<sup>&amp;</sup></b>
<b>Mortality</b>	<b>Time for border crossing</b>	<b>Numerical (No normal)</b>	<b>3/59</b>	<b>Wilcoxon test</b>	<b>0.021*</b>
<b>Mortality</b>	Temperature inside vehicle TOTAL (BG+TR)	Numerical (Normal)	2/97	t-test	1.000
<b>Mortality</b>	Humidity inside vehicle (BG+TR)	Numerical (No normal)	2/49	Wilcoxon test	0.176
<b>Mortality</b>	Inside THI (BG+TR)	Numerical (Normal)	5/63	t-test	0.589
<b>Mortality</b>	Heat stress scale (TR+BG)	Categorical	1/67	-	**
<b>Mortality</b>	<b>Bedding total (BG+TR)</b>	<b>Categorical</b>	<b>7/97</b>	<b>Fisher</b>	<b>0.004*</b>
<b>Mortality</b>	<b>Headspace total (BG+TR)</b>	<b>Categorical</b>	<b>7/97</b>	<b>Fisher</b>	<b>0.003*</b>
<b>Mortality</b>	<b>Loading density total (BG+TR)</b>	<b>Categorical</b>	<b>6/97</b>	<b>Fisher</b>	<b>0.027*</b>
<b>Mortality</b>	Correct separation mixed sizes total (BG+TR)	Categorical	2/35	Fisher	0.5045
<b>Mortality</b>	Correct separation mixed horn/hornless (BG+TR)	Categorical	2/29	Fisher	1.000
<b>Mortality</b>	<b>Drinking system total (BG+TR)</b>	<b>Categorical</b>	<b>6/94</b>	<b>Fisher</b>	<b>0.088</b>
<b>Mortality</b>	Ventilation total (BG+TR)	Categorical	2/98	Fisher	1.000

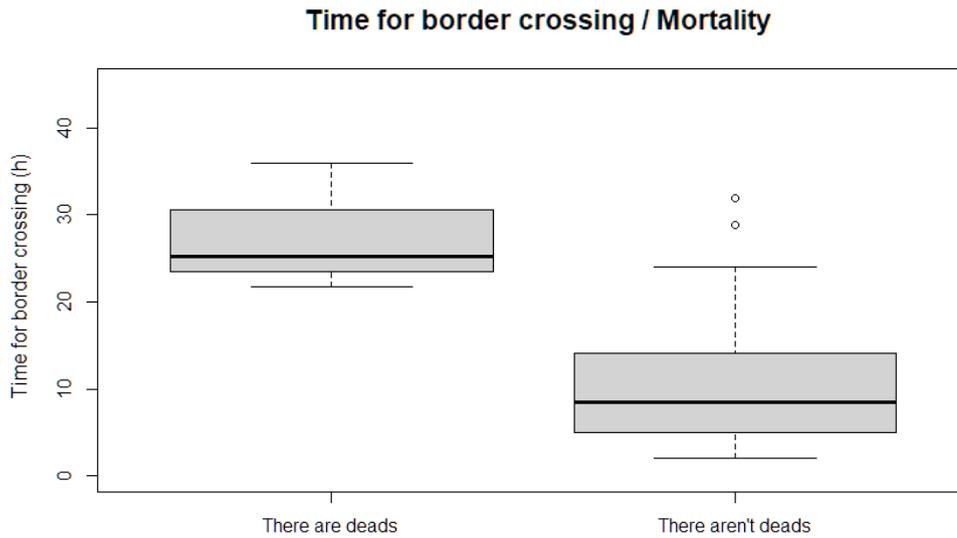
**bold with "\*\*" means p-value<0.05,**

**in bold means p-value <0.1,**

all the other cases p-value>0.1

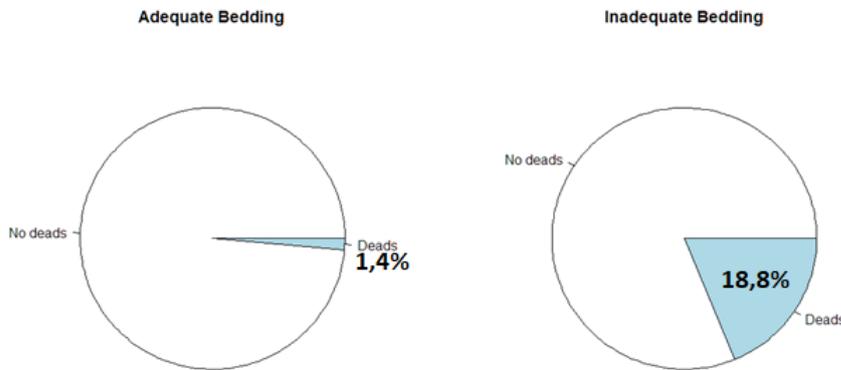
<sup>&</sup>Not considered;

\*\* Only data of non-dead



**Figure 5:** Effect of time for border crossing (hours) on the occurrence of dead cattle.

The highest numbers of *dead animals* were observed in the consignments that took 20 to 30 hours to cross the border between Bulgaria and Turkey. The more time the consignments waited to cross the borders, the more *dead animals* were observed, while no *dead animals* were observed in the consignments that needed 5 to 15 hours to cross the border<sup>3</sup>.



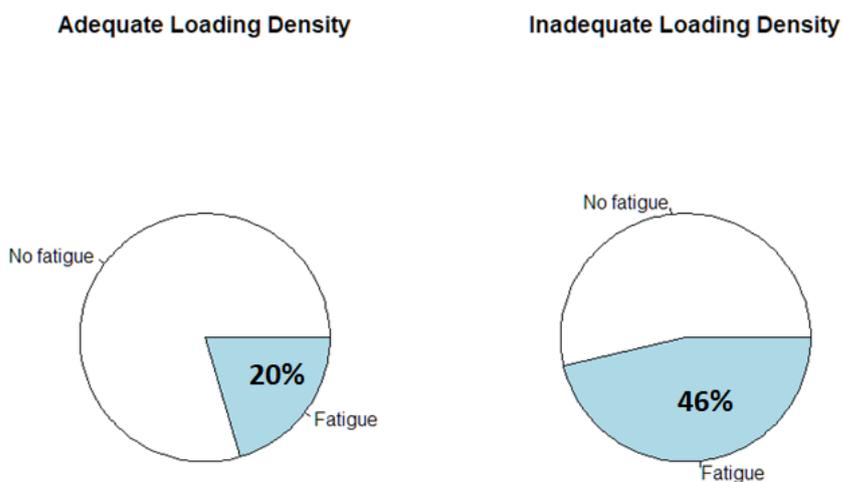
**Figure 6:** Effect of bedding adequacy on the incidence of dead cattle.

When EF bedding was considered *adequate*, *dead animals* were only found in **1.4%** of the consignments. When EF bedding was considered *inadequate*, the number of consignments with *dead animals* observed increased up to **18.8%**.

<sup>3</sup> Please note that only the first deck of the truck was inspected.

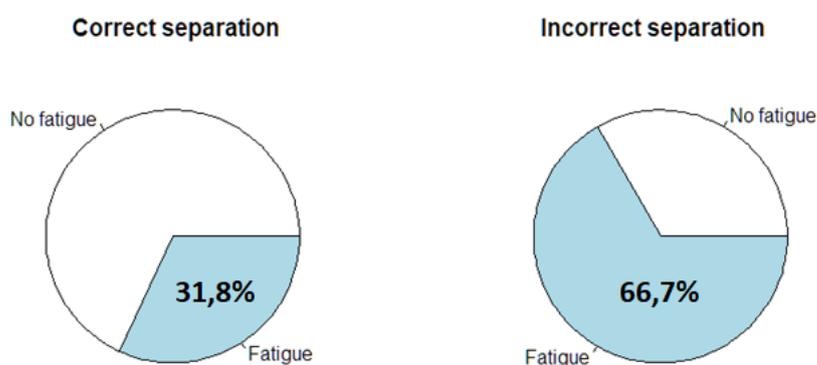
**Table 4: FATIGUE**

First variable (Categorical)	Second variable	Observation type (2 <sup>nd</sup> variable)	Number of observations in each group	Statistic test	p-value
Fatigue	Origin country	Categorical	31/69	Fisher	0.713
Fatigue	Distance to the place of the inspection	Numerical (No normal)	18/37	Wilcoxon test	0.121
Fatigue	Time for border crossing	Numerical (No normal)	22/40	Wilcoxon test	0.402
Fatigue	Temperature inside vehicle TOTAL (BG+TR)	Numerical (Normal)	29/70	t-test	0.684
Fatigue	Humidity inside a vehicle (BG+TR)	Numerical (No normal)	9/42	Wilcoxon test	0.653
Fatigue	Inside THI (BG+TR)	Numerical (Normal)	24/44	t-test	0.329
Fatigue	Heat stress scale (TR+BG)	Categorical	21/47	-	0.314
Fatigue	Bedding total (BG+TR)	Categorical	35/69	Fisher	0.743
Fatigue	Headspace total (BG+TR)	Categorical	31/61	Fisher	0.950
Fatigue	<b>Loading density total (BG+TR)</b>	<b>Categorical</b>	<b>35/68</b>	<b>Fisher</b>	<b>0.010*</b>
Fatigue	<b>Correct separation mixed sizes total (BG+TR)</b>	<b>Categorical</b>	<b>17/20</b>	<b>Fisher</b>	<b>0.049*</b>
Fatigue	Correct separation mixed horn/hornless (BG+TR)	Categorical	13/18	Fisher	1.000
Fatigue	Drinking system total (BG+TR)	Categorical	35/65	Fisher	0.323
Fatigue	Ventilation total (BG+TR)	Categorical	33/67	Fisher	0.574



**Figure 7: Effect of loading density on observed fatigue in consignments.**

When *loading density* was considered *adequate*, *fatigue* animals were observed in **20%** of the consignments. However, the number of *fatigue animals* observed increased to **46%** when *loading density* was considered *inadequate*.



**Figure 8:** Effect of the correct separation by size on observed fatigue in the consignments.

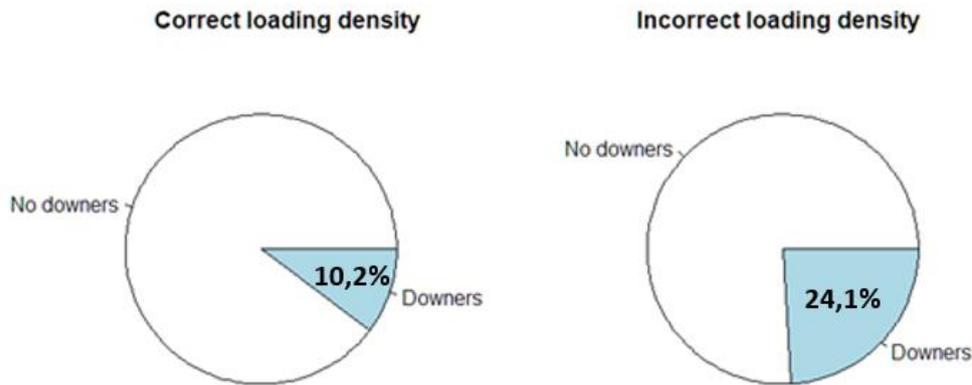
When the *separation* between animals was considered *adequate*, *fatigue animals* were found in nearly **32%** of the consignments. However, when the *separation* between animals was considered *inadequate* the number of *fatigue animals* increased to nearly **67%**.

**Table 5: DOWNER**

First variable (categorical)	Second variable	Observation type (2 <sup>nd</sup> variable)	Number of observations in each group	Statistical test	p-value
<b>Downer</b>	Origin country	Categorical	14/86	Fisher	0.179
<b>Downer</b>	Distance to destination	Numerical (No normal)	12/43	Wilcoxon test	0.007 <sup>&amp;</sup>
<b>Downer</b>	Time for border crossing	Numerical (No normal)	11/51	Wilcoxon test	0.868
<b>Downer</b>	Temperature inside vehicle TOTAL (BG+TR)	Numerical (Normal)	17/82	t-test	0.535
<b>Downer</b>	Humidity inside a vehicle (BG+TR)	Numerical (No normal)	12/39	Wilcoxon test	0.155
<b>Downer</b>	Inside THI (BG+TR)	Numerical (Normal)	12/56	t-test	0.190
<b>Downer</b>	Heat stress scale (TR+BG)	Categorical	15/53	-	0.303
<b>Downer</b>	<b>Bedding total (BG+TR)</b>	<b>Categorical</b>	<b>17/87</b>	<b>Fisher</b>	<b>0.043*</b>
<b>Downer</b>	Headspace total (BG+TR)	Categorical	18/74	Fisher	1.000
<b>Downer</b>	<b>Loading density total (BG+TR)</b>	<b>Categorical</b>	<b>18/85</b>	<b>Fisher</b>	<b>0.074</b>
<b>Downer</b>	Correct separation mixed sizes total (BG+TR)	Categorical	13/24	Fisher	1.000
<b>Downer</b>	Correct separation mixed horn/hornless (BG+TR)	Categorical	9/22	Fisher	0.220
<b>Downer</b>	Drinking system total (BG+TR)	Categorical	18/82	Fisher	1.000

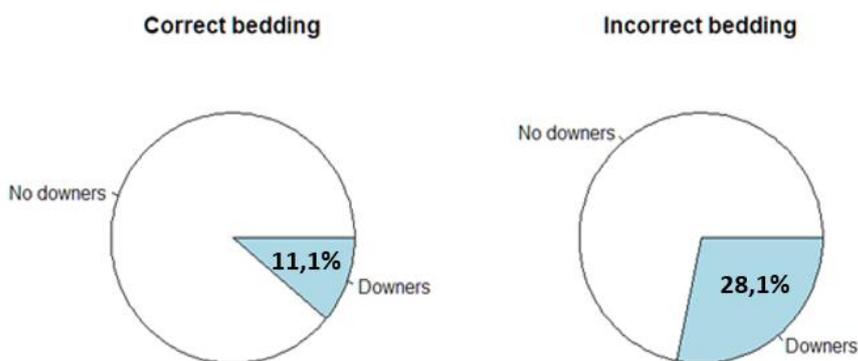
First variable (categorical)	Second variable	Observation type (2 <sup>nd</sup> variable)	Number of observations in each group	Statistical test	p-value
<b>Downer</b>	Ventilation total (BG+TR)	Categorical	17/83	Fisher	0.594

&Not considered



**Figure 9:** Effect of the correct loading density on observed downers in the consignments.

When *loading density* was considered *adequate*, *downer animals* were observed in **10.2%** of the consignments. However, the number of observed *downer animals* increased by up to **24%** when *loading density* was considered *inadequate*.



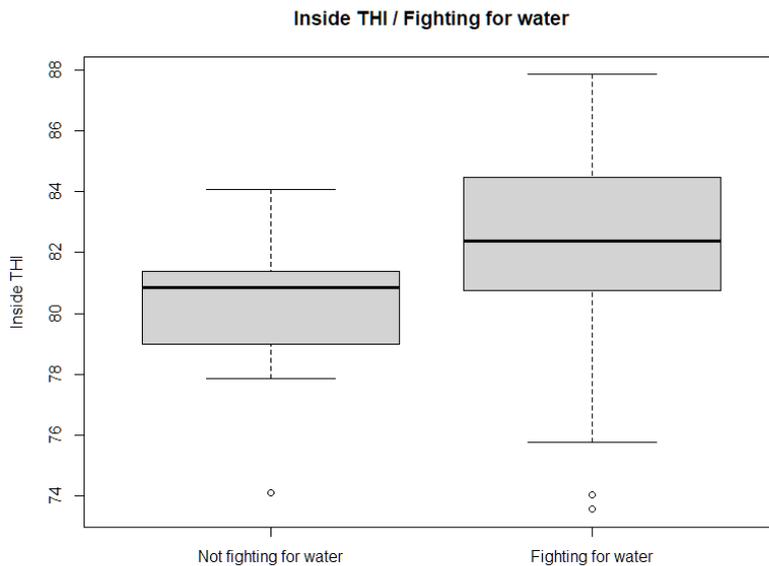
**Figure 10:** Effect of the correct bedding on observed downers in the consignments.

When *bedding* was considered *adequate*, *downer animals* were found in **11.1%** of the consignment. When *bedding* was considered *inadequate*, the number of consignments with observed downers increased by up to **28.1%**.

**Table 6: FIGHTING FOR WATER**

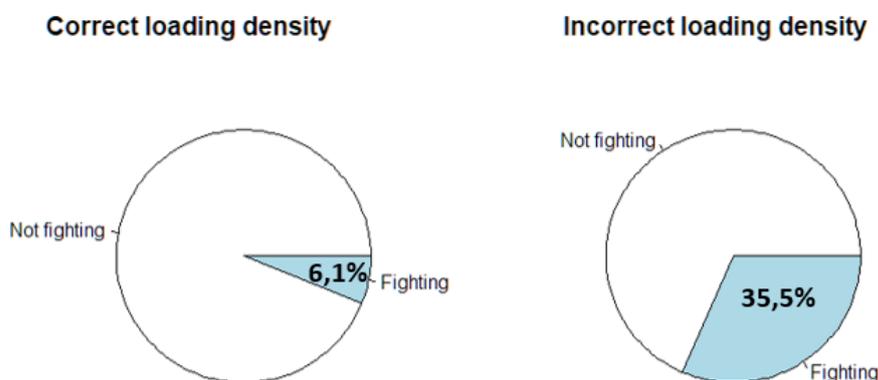
First variable (Categorical)	Second variable	Observation type (2 <sup>nd</sup> variable)	Number of observations in each group	Statistical test	p-value
<b>Fighting for water</b>	<b>Origin country</b>	<b>Categorical</b>	<b>17/83</b>	<b>Fisher</b>	<b>0.010*</b>
<b>Fighting for water</b>	Distance to the place of inspection	Numerical (No normal)	11/44	Wilcoxon test	0.027&
<b>Fighting for water</b>	Time for border crossing	Numerical (No normal)	11/51	Wilcoxon test	0.513
<b>Fighting for water</b>	Temperature inside vehicle TOTAL (BG+TR)	Numerical (Normal)	18/51	t-test	0.558
<b>Fighting for water</b>	Humidity inside vehicle (BG+TR)	Numerical (No normal)	6/45	Wilcoxon test	0.538
<b>Fighting for water</b>	<b>Inside THI (BG+TR)</b>	<b>Numerical (Normal)</b>	<b>11/57</b>	<b>t-test</b>	<b>0.017*</b>
<b>Fighting for water</b>	Heat stress scale (TR+BG)	Categorical	13/55	-	0.241
<b>Fighting for water</b>	Bedding total (BG+TR)	Categorical	19/85	Fisher	0.276
<b>Fighting for water</b>	Headspace total (BG+TR)	Categorical	16/76	Fisher	1.000
<b>Fighting for water</b>	<b>Loading density total (BG+TR)</b>	<b>Categorical</b>	<b>20/83</b>	<b>Fisher</b>	<b>0.001*</b>
<b>Fighting for water</b>	Correct separation mixed sizes total (BG+TR)	Categorical	16/21	Fisher	0.749
<b>Fighting for water</b>	Correct separation mixed horn/hornless (BG+TR)	Categorical	13/18	Fisher	0.433
<b>Fighting for water</b>	Drinking system total (BG+TR)	Categorical	18/82	Fisher	0.175
<b>Fighting for water</b>	Ventilation total (BG+TR)	Categorical	16/84	Fisher	0.411

&Not considered



**Figure 11: Effect of the THI on observed animals fighting for water.**

The highest number of animals *fighting for access to water* was observed when the *THI* parameter inside the vehicle ranged between 81 and 84.



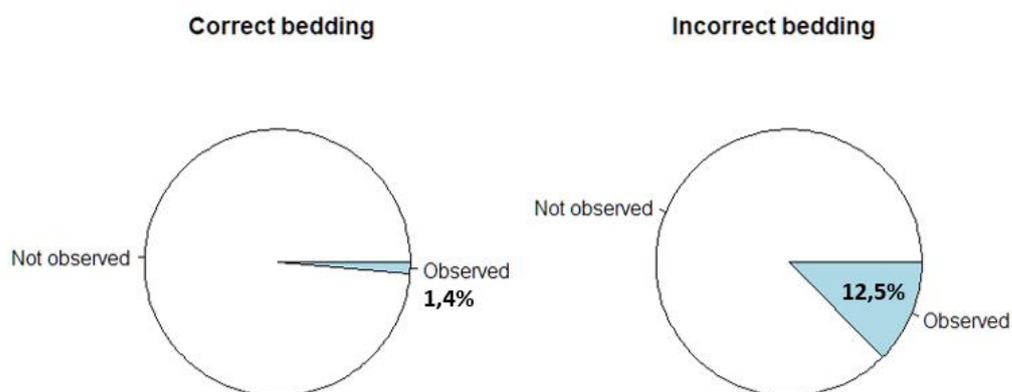
**Figure 12:** Effect of the correct loading density on observed animals fighting for water.

When *loading density* was considered *adequate*, animals *fighting for water* were observed in **6.1%** of the consignments. However, the number of observed animals *fighting for water* increased up to **35.5%** when *loading density* was considered *inadequate*.

**Table 7: DRINKING FROM URINE/EATING FILTHY/BEDDING**

First variable (Categorical)	Second variable	Observation type (2 <sup>nd</sup> variable)	Number of observations in each group	Statistical test	p-value
Drinking from urine/eating filthy	Origin country	Categorical	6/94	Fisher	0.561
Drinking from urine/eating filthy	Distance to the place of inspection	Numerical (No normal)	3/52	Wilcoxon test	0.188
Drinking from urine/eating filthy	Time for border crossing	Numerical (No normal)	4/58	Wilcoxon test	0.636
Drinking from urine/eating filthy	Temperature inside vehicle TOTAL (BG+TR)	Numerical (Normal)	4/95	t-test	1.000
Drinking from urine/eating filthy	Humidity inside vehicle (BG+TR)	Numerical (No normal)	2/49	Wilcoxon test	0.264
Drinking from urine/eating filthy	Inside THI (BG+TR)	Numerical (Normal)	3/65	t-test	0.370
Drinking from urine/eating filthy	Heat stress scale (TR+BG)	Categorical	2/66	-	1.000
Drinking from urine/eating filthy	<b>Bedding total (BG+TR)</b>	<b>Categorical</b>	<b>5/99</b>	<b>Fisher</b>	<b>0.030*</b>
Drinking from urine/eating filthy	Headspace total (BG+TR)	Categorical	1/91	Fisher	1.000

First variable (Categorical)	Second variable	Observation type (2 <sup>nd</sup> variable)	Number of observations in each group	Statistical test	p-value
Drinking from urine/eating filthy	Loading density total (BG+TR)	Categorical	4/99	Fisher	1.000
Drinking from urine/eating filthy	Correct separation mixed sizes total (BG+TR)	Categorical	0/37	Fisher	1.000
Drinking from urine/eating filthy	Correct separation mixed horn/hornless (BG+TR)	Categorical	0/31	Fisher	1.000
Drinking from urine/eating filthy	Drinking system total (BG+TR)	Categorical	6/94	Fisher	0.089
Drinking from urine/eating filthy	Ventilation total (BG+TR)	Categorical	5/95	Fisher	1.000



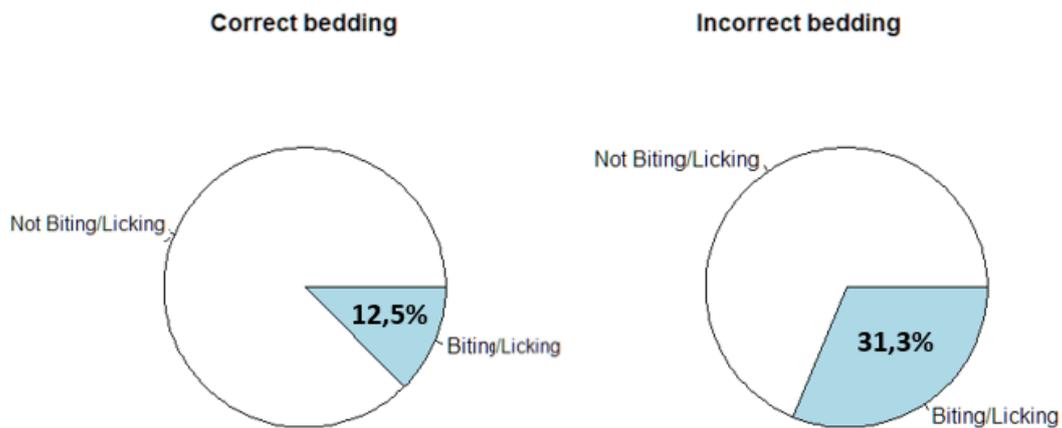
**Figure 13:** Effect of the bedding on observed animals drinking urine or eating filth in the consignments.

When *bedding* was considered *adequate*, animals drinking urine/eating filthy bedding were found in **1.4%** of the consignments. When *bedding* was considered *inadequate*, the number of consignments with observed animals drinking urine/eating filthy bedding increased up to **12.5%**.

**Table 8: BITING/LICKING BARS**

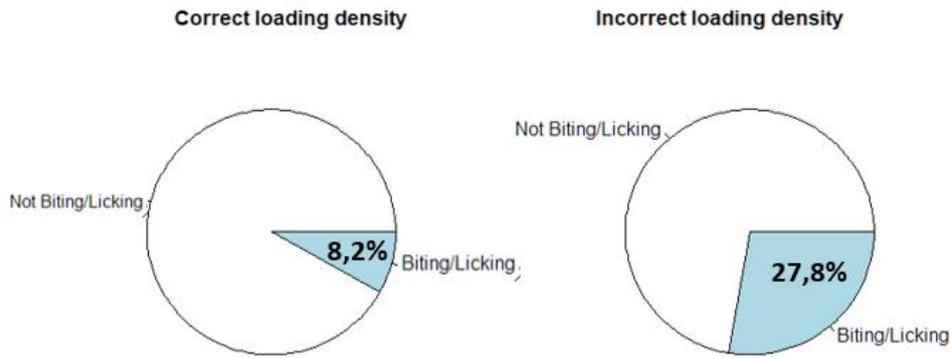
First variable (Categorical)	Second variable	Observation type (2 <sup>nd</sup> variable)	Number of observations in each group	Statistic test	p-value
Biting/licking bars	Origin country	Categorical	19/81	Fisher	0.429
Biting/licking bars	Distance to place of inspection	Numerical (No normal)	10/45	Wilcoxon test	0.141
Biting/licking bars	Time for border crossing	Numerical (No normal)	13/49	Wilcoxon test	0.903
Biting/licking bars	Temperature inside vehicle TOTAL (BG+TR)	Numerical (Normal)	16/83	t-test	1.000
Biting/licking bars	Humidity inside vehicle (BG+TR)	Numerical (No normal)	4/47	Wilcoxon test	0.265

First variable (Categorical)	Second variable	Observation type (2 <sup>nd</sup> variable)	Number of observations in each group	Statistic test	p-value
Biting/licking bars	Inside THI (BG+TR)	Numerical (Normal)	12/56	t-test	0.218
Biting/licking bars	Heat stress scale (TR+BG)	Categorical	9/59	-	0.520
Biting/licking bars	<b>Bedding total (BG+TR)</b>	<b>Categorical</b>	<b>19/85</b>	<b>Fisher</b>	<b>0.030*</b>
Biting/licking bars	Headspace total (BG+TR)	Categorical	14/78	Fisher	0.064
Biting/licking bars	<b>Loading density total (BG+TR)</b>	<b>Categorical</b>	<b>19/84</b>	<b>Fisher</b>	<b>0.012*</b>
Biting/licking bars	Correct separation mixed sizes total (BG+TR)	Categorical	7/30	Fisher	0.408
Biting/licking bars	Correct separation mixed horn/hornless (BG+TR)	Categorical	7/24	Fisher	0.384
Biting/licking bars	Drinking system total (BG+TR)	Categorical	20/80	Fisher	0.307
Biting/licking bars	Ventilation total (BG+TR)	Categorical	14/86	Fisher	0.090



**Figure 14:** Effect of bedding on observed animals biting/licking bars in the consignments.

When *bedding* was considered *adequate*, *animals biting/licking* metal bars of the vehicle were found in **12.5%** of the consignments. When *bedding* was considered *inadequate*, the number of consignments with observed *animals biting/licking* metal bars of the vehicle increased up to **31.3%**.

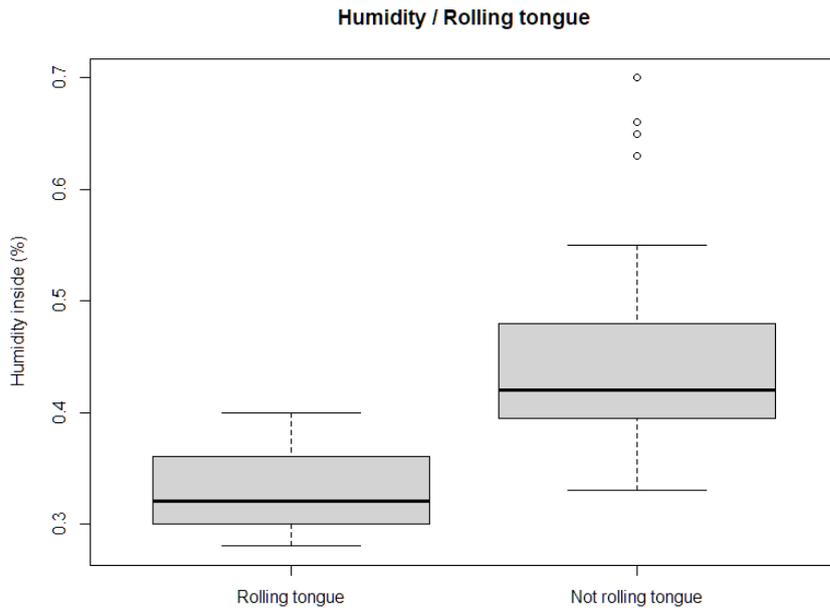


**Figure 15:** Effect of the loading density on observed animals biting/licking bars in the consignments.

When *loading density* was considered *adequate*, animals biting/licking the metal bars of vehicle were observed in **8.2%** of the consignments. However, the number of observed *animal biting/licking* the metal bars of vehicle increased up to **27.8%** when *loading density* was considered *inadequate*.

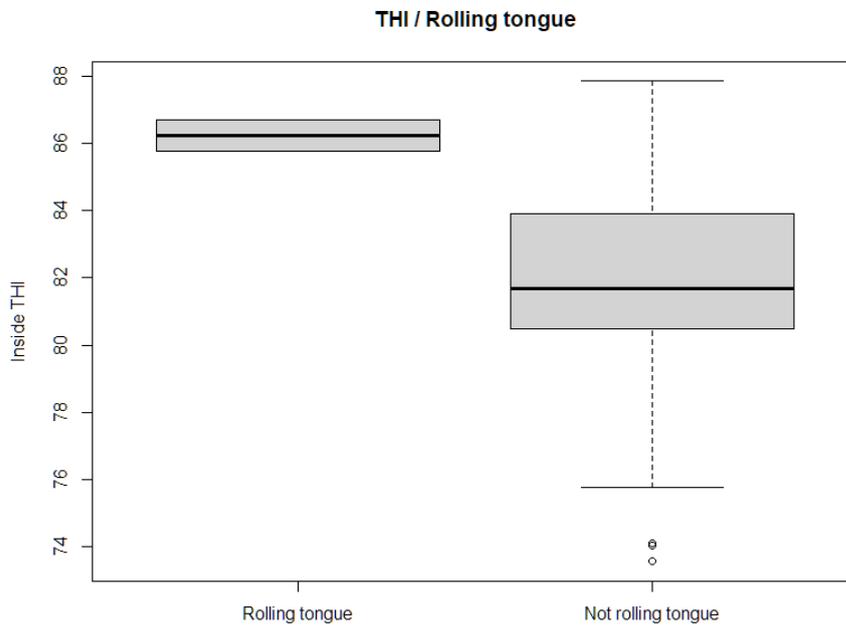
**Table 9: ROLLING TONGUES**

First variable (Categorical)	Second variable	Observation type (2 <sup>nd</sup> variable)	Number of observations in each group	Statistical test	p-value
Rolling tongues	Origin country	Categorical	9/91	Fisher	0.667
Rolling tongues	Distance to destination	Numerical (No normal)	5/50	Wilcoxon test	0.692
Rolling tongues	Time for border crossing	Numerical (No normal)	6/56	Wilcoxon test	0.198
Rolling tongues	Temperature inside vehicle TOTAL (BG+TR)	Numerical (Normal)	9/90	t-test	1.000
Rolling tongues	<b>Humidity inside vehicle (BG+TR)</b>	<b>Numerical (No normal)</b>	<b>4/47</b>	<b>Wilcoxon test</b>	<b>0.005*</b>
Rolling tongues	<b>Inside THI (BG+TR)</b>	<b>Numerical (Normal)</b>	<b>2/66</b>	<b>t-test</b>	<b>0.040*</b>
Rolling tongues	Heat stress scale (TR+BG)	Categorical	6/62	-	0.379
Rolling tongues	<b>Bedding total (BG+TR)</b>	<b>Categorical</b>	<b>9/95</b>	<b>Fisher</b>	<b>0.023*</b>
Rolling tongues	Headspace total (BG+TR)	Categorical	9/83	Fisher	0.187
Rolling tongues	Loading density total (BG+TR)	Categorical	9/94	Fisher	0.493
Rolling tongues	Correct separation mixed sizes total (BG+TR)	Categorical	3/34	Fisher	1.000
Rolling tongues	Correct separation mixed horn/hornless (BG+TR)	Categorical	2/29	Fisher	0.503
Rolling tongues	Drinking system total (BG+TR)	Categorical	8/92	Fisher	0.255
Rolling tongues	Ventilation total (BG+TR)	Categorical	7/93	Fisher	1.000



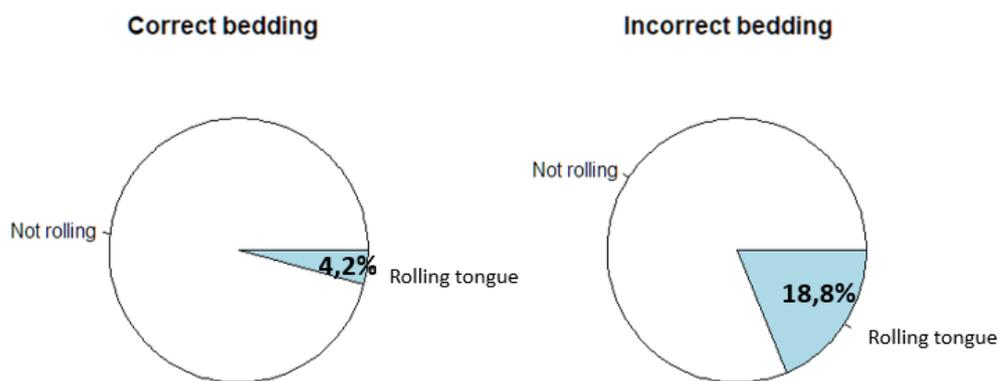
**Figure 16:** Effect of the humidity on the presence of animals with rolling tongues.

Most animals that *rolled their tongues* were observed when the *humidity* inside the vehicle ranged between 0.3 and 0.35.



**Figure 17:** Effect of the THI on the presence of animals with rolling tongues.

Most animals that *rolled their tongues* were observed when the *THI* parameter inside the vehicle ranged around 86.

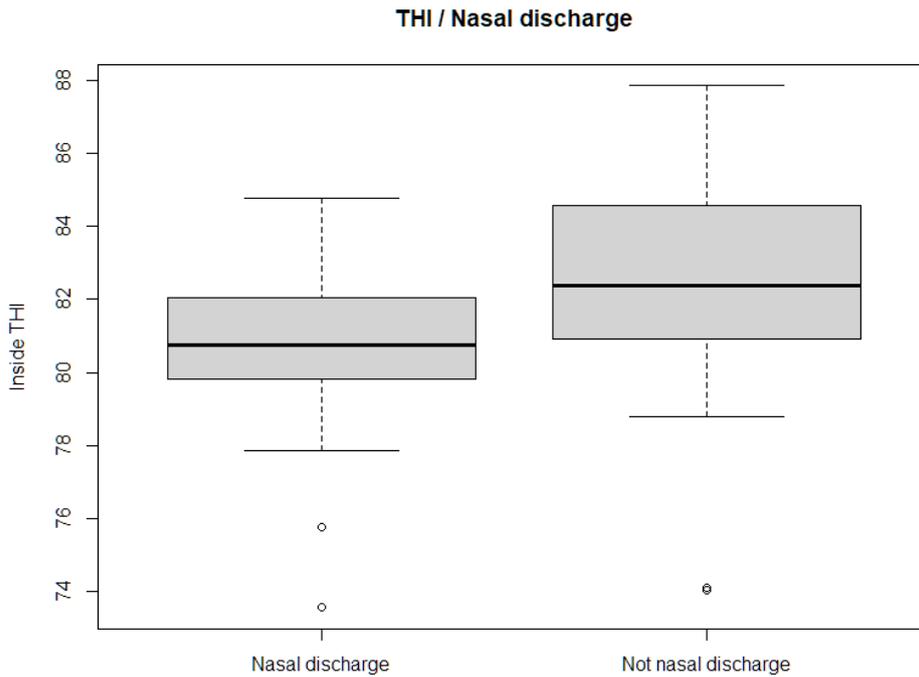


**Figure 18:** Effect of bedding on tongue rolling of observed animals.

When *bedding* was considered *adequate*, tongue rolling of *animals* was observed in **4.2%** of the consignment. When *bedding* was considered *inadequate*, the number of consignments with observed tongue-rolling animals raised up to **18.8%**.

**Table 10: NASAL DISCHARGE**

First variable (Categorical)	Second variable	Observation type (2 <sup>nd</sup> variable)	Number of observations in each group	Statistical test	p-value
<b>Nasal discharge</b>	Origin country	Categorical	29/71	Fisher	0.648
<b>Nasal discharge</b>	Distance to the point of inspection	Numerical (No normal)	5/48	Wilcoxon test	0.177
<b>Nasal discharge</b>	Time for border crossing	Numerical (No normal)	6/56	Wilcoxon test	0.200
<b>Nasal discharge</b>	Temperature inside vehicle TOTAL (BG+TR)	Numerical (Normal)	30/69	t-test	0.687
<b>Nasal discharge</b>	Humidity inside vehicle (BG+TR)	Numerical (No normal)	13/38	Wilcoxon test	0.369
<b>Nasal discharge</b>	<b>Inside THI (BG+TR)</b>	<b>Numerical (Normal)</b>	<b>19/49</b>	<b>t-test</b>	<b>0.007*</b>
<b>Nasal discharge</b>	Heat stress scale (TR+BG)	Categorical	19/49	-	0.614
<b>Nasal discharge</b>	Bedding total (BG+TR)	Categorical	34/70	Fisher	0.119
<b>Nasal discharge</b>	Headspace total (BG+TR)	Categorical	31/61	Fisher	0.564
<b>Nasal discharge</b>	Loading density total (BG+TR)	Categorical	33/70	Fisher	0.834
<b>Nasal discharge</b>	Correct separation mixed sizes total (BG+TR)	Categorical	15/22	Fisher	1.000
<b>Nasal discharge</b>	Correct separation mixed horn/hornless (BG+TR)	Categorical	11/20	Fisher	0.429
<b>Nasal discharge</b>	Drinking system total (BG+TR)	Categorical	34/66	Fisher	0.382
<b>Nasal discharge</b>	Ventilation total (BG+TR)	Categorical	34/66	Fisher	1.000



**Figure 19:** Effect of the THI on observed nasal discharge.

Most animals with *nasal discharge* were observed when the *THI* parameter inside the vehicle ranged between 80 and 82.

**Table 11: CATTLE SWEATING**

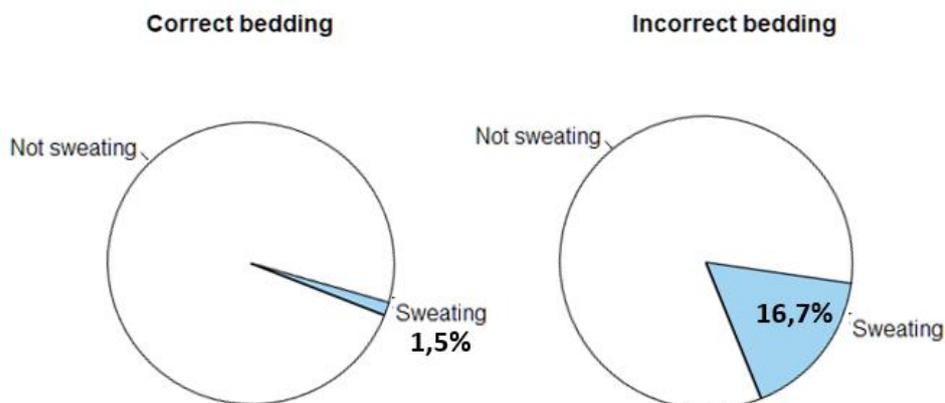
First variable (Categorical)	Second variable	Observation type (2 <sup>nd</sup> variable)	Number of observations in each group	Statistic test	p-value
Cattle sweating	Origin country	Categorical	5/74	Fisher	0.016*
Cattle sweating	Distance to the point of inspection	Numerical (No normal)	3/41	Wilcoxon test	0.051
Cattle sweating	Time for border crossing	Numerical (No normal)	1/46	Wilcoxon test	0.012*
Cattle sweating	Temperature inside vehicle TOTAL (BG+TR)	Numerical (Normal)	1/82	t-test	1.000
Cattle sweating	Humidity inside the vehicle (BG+TR)	Numerical (No normal)	1/50	Wilcoxon test	0.118
Cattle sweating	Inside THI (BG+TR)	Numerical (Normal)	2/53	t-test	0.982
Cattle sweating	Heat stress scale (TR+BG)	Categorical	1/67	-	1.000
Cattle sweating	Bedding total (BG+TR)	Categorical	4/80	Fisher	0.029*
Cattle sweating	Headspace total (BG+TR)	Categorical	5/72	Fisher	0.196
Cattle sweating	Loading density total (BG+TR)	Categorical	5/80	Fisher	0.360
Cattle sweating	Correct separation mixed sizes total (BG+TR)	Categorical	3/34	Fisher	0.257

First variable (Categorical)	Second variable	Observation type (2 <sup>nd</sup> variable)	Number of observations in each group	Statistic test	p-value
Cattle sweating	Correct separation mixed horn/hornless(BG+TR)	Categorical	3/28	Fisher	1.000
Cattle sweating	Drinking system total (BG+TR)	Categorical	3/77	Fisher	1.000
Cattle sweating	Ventilation total (BG+TR)	Categorical	2/81	Fisher	1.000

**Table 12:** Sweating animals depending on the country of origin.

Sweating	NOT FOUND	YES	%YES
AUSTRIA	6	1	14%
CZECHIA	27	1	4%
DENMARK	2	0	0%
GERMANY	9	0	0%
HUNGARY	12	0	0%
LATVIA	1	0	0%
LITHUANIA	1	2	67%
ROMANIA	3	1	25%
SLOVAKIA	13	0	0%

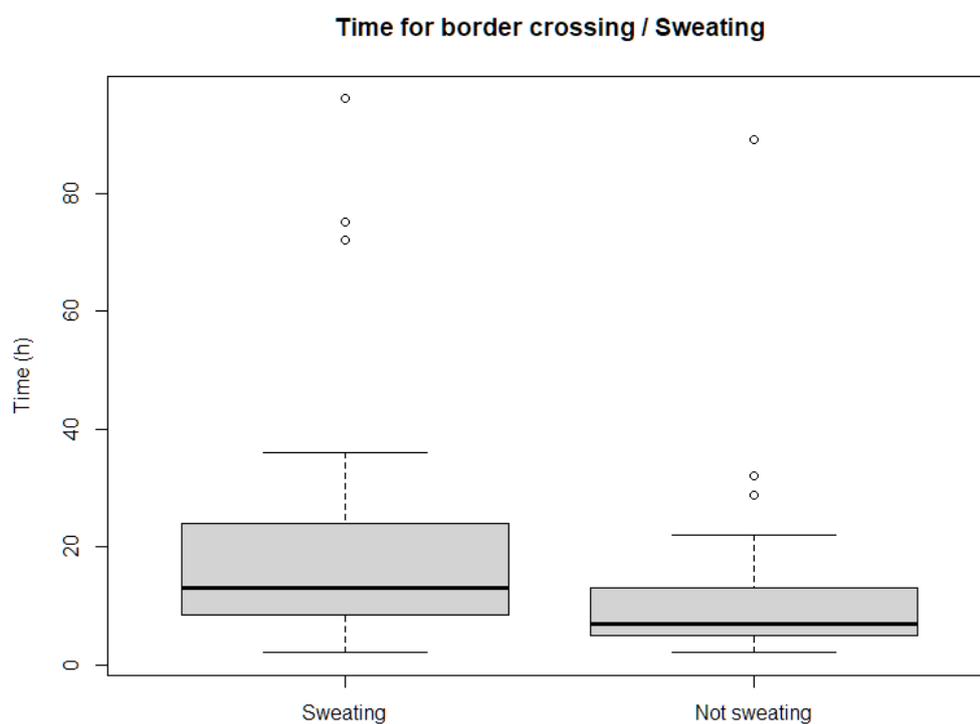
In 67% of consignments coming from Lithuania and 25% of consignments coming from Romania animals with *coats covered with sweat* were observed. Also, in animals coming from Austria, a high percentage (14%) of sweaty animals was observed.



**Figure 20:** Effect of bedding on observed sweating.

When *bedding* was considered *adequate*, *sweating animals* were observed in **1.5%** of the consignments. When *bedding* was considered *inadequate*, the number of consignments with observed sweating animals raised up to **16.7%**.

**Figure 21:** Effect of time for border crossing on observed sweating animals.



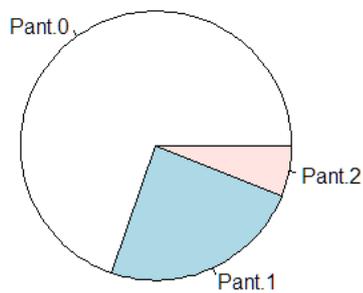
Most *sweating animals* were observed when the vehicle took 10 to 25 hours to *cross the border*.

**Table 13: CATTLE PANTING SCORE**

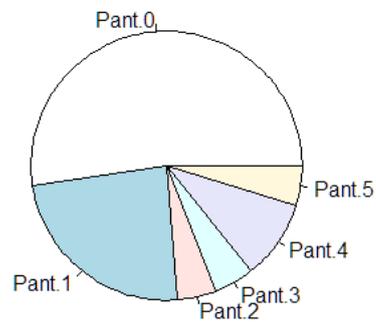
First variable (Categorical)	Second variable	Observation type (2 <sup>nd</sup> variable)	Number of observations in each group	Statistical test	p-value
<b>Cattle sweating</b>	Origin country	Categorical	-	Fisher	0.191
<b>Cattle sweating</b>	Distance to destination	Numerical (No normal)	-	Wilcoxon test	0.101
<b>Cattle sweating</b>	Time for border crossing	Numerical (No normal)	-	Wilcoxon test	0.250
<b>Cattle sweating</b>	Temperature inside vehicle TOTAL (BG+TR)	Numerical (Normal)	-	t-test	0.528
<b>Cattle sweating</b>	Humidity inside vehicle (BG+TR)	Numerical (No normal)	-	Wilcoxon test	0.540
<b>Cattle sweating</b>	Inside THI (BG+TR)	Numerical (Normal)	-	t-test	0.593
<b>Cattle sweating</b>	Heat stress scale (TR+BG)	Categorical	-	-	0.224
<b>Cattle sweating</b>	<b>Bedding total (BG+TR)</b>	<b>Categorical</b>	-	<b>Fisher</b>	<b>0.022*</b>
<b>Cattle sweating</b>	<b>Headspace total (BG+TR)</b>	<b>Categorical</b>	-	<b>Fisher</b>	<b>0.033*</b>
<b>Cattle sweating</b>	Loading density total (BG+TR)	Categorical	-	Fisher	0.239
<b>Cattle sweating</b>	Correct separation mixed sizes total (BG+TR)	Categorical	-	Fisher	0.193

First variable (Categorical)	Second variable	Observation type (2 <sup>nd</sup> variable)	Number of observations in each group	Statistical test	p-value
<b>Cattle sweating</b>	Correct separation mixed horn/hornless(BG+TR)	Categorical	-	Fisher	1.000
<b>Cattle sweating</b>	Drinking system total (BG+TR)	Categorical	-	Fisher	0.752
<b>Cattle sweating</b>	Ventilation total (BG+TR)	Categorical	-	Fisher	0.343

**Adequate bedding**



**Inadequate bedding**



**Figure 3:** Effect of adequate/inadequate bedding on the panting score of cattle.

When *bedding* was considered *adequate*, panting animals of scores 1 and 2 were found in nearly **30%** of the consignments. When *bedding* was considered *inadequate*, the number of panting animals increased up to **45%** with a panting score between 1 to 5.

## 6. ANNEX5. DETAILED RESULTS FOR THE DIFFERENT

**Table A1:** Correlations between ABIs and EFs including the specific *p-value* for each analysis.

EF \ ABI	Mortality	Fatigue	Downer unfit	Fighting for water	Drinking Urine / Eating filthy	Biting/ Licking bars	Rolling tongue	Nasal Discharge	Sweating	Panting Score	Number of ABIs affected
Bedding total (BG+TR)	0.004	0.743	0.043	0.276	0.030	0.030	0.023	0.119	0.029	0.022	7
Loading density total (BG+TR)	0.027	0.01	0.074	0.001	1.000	0.012	0.493	0.834	0.360	0.239	5
Inside THI TOTAL (BG+TR)	0.589	0.329	0.19	0.017	0.370	0.218	0.040	0.007	0.982	0.593	3
Origin country	0.098	0.713	0.179	0.01	0.561	0.429	0.667	0.648	0.016	0.191	2
Time for border crossing	0.021	0.402	0.868	0.513	0.636	0.903	0.198	0.200	0.012	0.250	2
Headspace total (BG+TR)	0.003	0.95	1	1	1.000	0.064	0.187	0.564	0.196	0.033	2
Humidity inside vehicle TOTAL (BG+TR)	0.176	0.653	0.155	0.538	0.264	0.265	0.005	0.369	0.118	0.540	1
Correct separation mixed sizes total (BG+TR)	0.5045	0.049	1	0.749	1.000	0.408	1.000	1.000	0.257	0.193	1
Temperature inside vehicle TOTAL (BG+TR)	1	0.684	0.535	0.558	1.000	1.000	1.000	0.687	1.000	0.528	0
Heat stress scale (TR+BG)	1	0.314	0.303	0.241	1.000	0.520	0.379	0.614	1.000	0.224	0
Correct separation mixed horn/hornless(BG+TR)	1	1	0.22	0.433	1.000	0.384	0.503	0.429	1.000	1.000	0
Drinking system total (BG+TR)	0.088	0.323	1	0.175	0.089	0.307	0.255	0.382	1.000	0.752	0
Ventilation total (BG+TR)	1	0.574	0.594	0.411	1.000	0.090	1.000	1.000	1.000	0.343	0

## ADDITIONAL TABLES ORDERED BY EXTERNAL EFFECT

The following tables describe the same information as in the previous section, but in a different format to provide a different type of visualisation.

**Table B1. Bedding total.**

ABI	Number of observations in each group (YES/NO)	p-value	
<b>Mortality</b>	<b>7/97</b>	<b>0.004</b>	*
Fatigue	35/69	0.743	
<b>Downer unfit</b>	<b>17/87</b>	<b>0.043</b>	*
Fighting for water	19/85	0.276	
<b>Drinking from urine/eating filthy</b>	<b>5/99</b>	<b>0.030</b>	*
<b>Biting/licking bars</b>	<b>19/85</b>	<b>0.030</b>	*
<b>Rolling tongue</b>	<b>9/95</b>	<b>0.023</b>	*
Nasal discharge	34/70	0.119	
<b>Sweating</b>	<b>4/80</b>	<b>0.029</b>	*
<b>Panting score</b>	-	<b>0.022</b>	*

ABIs in bold and with "\*" means p-value<0.05, ABIs in bold means p-value <0.1, all the other cases p-value>0.1

**Table B2. Loading density.**

ABI	Number of observations in each group (YES/NO)	p-value	
<b>Mortality</b>	<b>6/97</b>	<b>0.027</b>	*
<b>Fatigue</b>	<b>35/68</b>	<b>0.010</b>	*
<b>Downer unfit</b>	<b>18/85</b>	<b>0.074</b>	
<b>Fighting for water</b>	<b>20/83</b>	<b>0.001</b>	*
<b>Drinking from Urine/Eating filthy</b>	4/99	1.000	
<b>Biting/Licking bars</b>	<b>19/84</b>	<b>0.012</b>	*
Rolling tongue	9/94	0.493	
Nasal Discharge	33/70	0.834	
Sweating	5/80	0.360	
<b>Panting Score</b>	-	0.239	

ABIs in bold and with "\*" means p-value<0.05, ABIs in bold means p-value <0.1, all the other cases p-value>0.1

**Table B3. Inside THI.**

ABI	Number of observations in each group (YES/NO)	p-value	
<b>Mortality</b>	5/63	0.589	
<b>Fatigue</b>	24/44	0.329	
<b>Downer unfit</b>	12/56	0.190	
<b>Fighting for water</b>	<b>11/57</b>	<b>0.017</b>	*
<b>Drinking from Urine/Eating filthy</b>	3/65	0.370	
<b>Biting/Licking bars</b>	12/56	0.218	
<b>Rolling tongue</b>	<b>2/66</b>	<b>0.040</b>	*
<b>Nasal Discharge</b>	<b>19/49</b>	<b>0.007</b>	*
Sweating	2/53	0.982	
<b>Panting Score</b>	-	0.593	

ABIs in bold and with "\*" means p-value<0.05, ABIs in bold means p-value <0.1, all the other cases p-value>0.1

Table B4. Origin country.

ABI	Number of observations in each group (YES/NO)	p-value	
<b>Mortality</b>	<b>3/91</b>	<b>0.098</b>	
Fatigue	31/69	0.713	
Downer unfit	14/86	0.179	
<b>Fighting for water</b>	<b>17/83</b>	<b>0.010</b>	*
Drinking from Urine/Eating filthy	6/94	0.561	
Biting/Licking bars	19/81	0.429	
Rolling tongue	9/91	0.667	
Nasal Discharge	29/71	0.648	
<b>Sweating</b>	<b>5/74</b>	<b>0.016</b>	*
Panting Score	-	0.191	

ABIs in bold and with "\*" means p-value<0.05, ABIs in bold means p-value <0.1, all the other cases p-value>0.1

Table B5. Time for border crossing.

ABI	Number of observations in each group (YES/NO)	p-value	
<b>Mortality</b>	<b>3/59</b>	<b>0.021</b>	*
Fatigue	22/40	0.402	
Downer unfit	11/51	0.868	
Fighting for water	11/51	0.513	
Drinking from Urine/Eating filthy	4/58	0.636	
Biting/Licking bars	13/49	0.903	
Rolling tongue	6/56	0.198	
Nasal Discharge	6/56	0.200	
<b>Sweating</b>	<b>1/46</b>	<b>0.012</b>	*
Panting Score	-	0.250	

ABIs in bold and with "\*" means p-value<0.05, ABIs in bold means p-value <0.1, all the other cases p-value>0.1

Table B6. Headspace total.

ABI	Number of observations in each group (YES/NO)	p-value	
<b>Mortality</b>	<b>7/97</b>	<b>0.003</b>	*
Fatigue	31/61	0.950	
Downer unfit	18/74	1.000	
Fighting for water	16/76	1.000	
Drinking from Urine/Eating filthy	1/91	1.000	
<b>Biting/Licking bars</b>	<b>14/78</b>	<b>0.064</b>	
Rolling tongue	9/83	0.187	
Nasal Discharge	31/61	0.564	
Sweating	5/72	0.196	
<b>Panting Score</b>	-	<b>0.033</b>	*

ABIs in bold and with "\*" means p-value<0.05, ABIs in bold means p-value <0.1, all the other cases p-value>0.1

**Table B7. Humidity inside vehicle.**

ABI	Number of observations in each group (YES/NO)	p-value	
Mortality	2/49	0.176	
Fatigue	9/42	0.653	
Downer unfit	12/39	0.155	
Fighting for water	6/45	0.538	
Drinking from Urine/Eating filthy	2/49	0.264	
Biting/Licking bars	4/47	0.265	
Rolling tongue	<b>4/47</b>	<b>0.005</b>	*
Nasal Discharge	13/38	0.369	
Sweating	1/50	0.118	
Panting Score	-	0.54	

ABIs in bold and with "\*" means p-value<0.05, ABIs in bold means p-value <0.1, all the other cases p-value>0.1

**Table B8. Correct separation mixed sizes.**

ABI	Number of observations in each group (YES/NO)	p-value	
Mortality	2/35	0.505	
Fatigue	<b>17/20</b>	<b>0.049</b>	*
Downer unfit	13/24	1.000	
Fighting for water	16/21	0.749	
Drinking from Urine/Eating filthy	0/37	1.000	
Biting/Licking bars	7/30	0.408	
Rolling tongue	3/34	1.000	
Nasal Discharge	<b>15/22</b>	1.000	
Sweating	<b>3/34</b>	0.257	
Panting Score	-	0.193	

ABIs in bold and with "\*" means p-value<0.05, ABIs in bold means p-value <0.1, all the other cases p-value>0.1

**Table B9. Temperature inside the vehicle.**

ABI	Number of observations in each group (YES/NO)	p-value	
Mortality	2/97	1.000	
Fatigue	29/70	0.684	
Downer unfit	17/82	0.535	
Fighting for water	18/51	0.558	
Drinking from Urine/Eating filthy	4/95	1.000	
Biting/Licking bars	16/83	1.000	
Rolling tongue	9/90	1.000	
Nasal Discharge	30/69	0.687	
Sweating	1/82	1.000	
Panting Score	-	0.528	

ABIs in bold and with "\*" means p-value<0.05, ABIs in bold means p-value <0.1, all the other cases p-value>0.1

**Table B10. Heat stress scale.**

ABI	Number of observations in each group (YES/NO)	p-value
<b>Mortality</b>	1/67	1.000
<b>Fatigue</b>	21/47	0.314
<b>Downer unfit</b>	15/53	0.303
<b>Fighting for water</b>	13/55	0.241
<b>Drinking from Urine/Eating filthy</b>	2/66	1.000
<b>Biting/Licking bars</b>	9/59	0.520
<b>Rolling tongue</b>	6/62	0.379
<b>Nasal Discharge</b>	19/49	0.614
<b>Sweating</b>	1/67	1.000
<b>Panting Score</b>	-	0.224

ABIs in bold and with "\*" means p-value<0.05, ABIs in bold means p-value <0.1, all the other cases p-value>0.1

**Table B11. Correct separation horn/hornless.**

ABI	Number of observations in each group (YES/NO)	p-value
<b>Mortality</b>	2/29	1.000
<b>Fatigue</b>	13/18	1.000
<b>Downer unfit</b>	9/22	0.220
<b>Fighting for water</b>	13/18	0.433
<b>Drinking from Urine/Eating filthy</b>	0/31	1.000
<b>Biting/Licking bars</b>	7/24	0.384
<b>Rolling tongue</b>	2/29	0.503
<b>Nasal Discharge</b>	11/20	0.429
<b>Sweating</b>	3/28	1.000
<b>Panting Score</b>	-	1.000

ABIs in bold and with "\*" means p-value<0.05, ABIs in bold means p-value <0.1, all the other cases p-value>0.1

**Table B12. Drinking system.**

ABI	Number of observations in each group (YES/NO)	p-value
<b>Mortality</b>	<b>6/94</b>	<b>0.088</b>
<b>Fatigue</b>	35/65	0.323
<b>Downer unfit</b>	18/82	1.000
<b>Fighting for water</b>	18/82	0.175
<b>Drinking from Urine/Eating filthy</b>	<b>6/94</b>	<b>0.089</b>
<b>Biting/Licking bars</b>	20/80	0.307
<b>Rolling tongue</b>	8/92	0.255
<b>Nasal Discharge</b>	34/66	0.382
<b>Sweating</b>	3/77	1.000
<b>Panting Score</b>	-	0.752

ABIs in bold and with "\*" means p-value<0.05, ABIs in bold means p-value <0.1, all the other cases p-value>0.1

**Table B13. Ventilation system.**

ABI	Number of observations in each group (YES/NO)	p-value	
<b>Mortality</b>	2/98	1.000	
<b>Fatigue</b>	33/67	0.574	
<b>Downer unfit</b>	17/83	0.594	
<b>Fighting for water</b>	16/84	0.411	
<b>Drinking from Urine/Eating filthy</b>	5/95	1.000	
<b>Biting/Licking bars</b>	<b>14/86</b>	<b>0.090</b>	
<b>Rolling tongue</b>	7/93	1.000	
<b>Nasal Discharge</b>	34/66	1.000	
<b>Sweating</b>	2/81	1.000	
<b>Panting Score</b>	-	0.343	

ABIs in bold and with "\*" means p-value<0.05, ABIs in bold means p-value <0.1, all the other cases p-value>0.1

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