# Boran cattle mortality due to ECF in relation to environmental factors

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# Abstract

ECF is a tick-borne disease caused by the protozoan parasite *Theileria parva parva*. It is responsible for many cattle falling ill and dying each year in Africa, affecting the farmers both financially and emotionally. The number of cattle dying due to ECF is estimated to be about 1 million head each year and the disease causes an economic burden of about half a billion U.S. dollars. To reduce the occurrence of ECF prophylactic treatment directed towards the ticks is used. Understanding what factors primarily affect the ticks and secondarily the disease is of great importance to prevent and fight the disease. In this study precipitation, biomass, dew and humidity was analysed together with mortality data for ECF. The environmental factors were chosen due to their likelihood to affect ticks crucial for spreading ECF. This study was conducted at the Ol Pejeta Conservancy in Laikipia, Kenya with data from the years 2016-2023. By examining these parameters, the goal was to identify environmental risk factors that can be used in the work to fight and prevent the disease. Results obtained by analysing the data showed a positive connection between precipitation, humidity, dew and biomass. Higher values of these environmental factors were connected to cattle dying due to ECF. Daily minimum, mean and maximum temperatures were also analysed, but no connections to death due to ECF were found.

*Keywords:* ECF, East Coast Fever, TBD, environment, precipitation, humidity, dew, biomass, grass height

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# Abbreviations

| | |
|---|---|
| ECF | East Coast Fever |
| OPC | Ol Pejeta Conservancy |
| TBD | Tick-borne disease |
| SLU | Swedish University of Agricultural Sciences |
| FMD | Foot and mouth disease |

# 1. Introduction

East coast fever is a disease responsible for severe economic losses of cattle in Africa. It is a tick-borne disease caused by the protozoan parasite *Theileria parva parva* and is primarily spread by its main vector, the brown ear tick *Rhipicephalus appendiculatus* (Maxie 2016). Acute disease causes fever, enlarged lymph nodes, depression, red blood cell parasitaemia and anaemia. In addition to the animal suffering inflicted by the disease there is also a financial impact due to high mortality, reduced growth and reduced production (Olwoch *et al.* 2008). The economic losses are difficult to estimate, and the figures vary between different sources. According to one source the economic burden is estimated to be about half a billion U.S. dollars per year and the disease kills at least 1 million cattle annually (Surve *et al.* 2023).

The aim of this study was to identify environmental risk factors for ECF focusing on the ticks that spread the disease.

This was done by using daily mortality reports from cattle (2016-2023) from Ol Pejeta Conservancy. OPC holds about 6000 Boran cattle making them the largest cattle farm in Africa (Ol Pejeta Conservancy n.d.). The cattle are integrated with the wildlife which optimises use of the grasslands but also raises challenges such as predation and tick-borne diseases. The mortality data was analysed together with environmental factors such as precipitation, biomass, humidity, dew and temperature. The reliability of the ECF diagnosis was also examined in relation to the diagnosis pneumonia which has similar symptoms, and both occur at OPC.

# 2. Literature review

## 2.1 Theileriosis

*Theileria spp*. are protozoan parasites of the order *Piroplasmida*, family *Theileriidae* (Zachary 2017). Speciation within the genus is not precise and the system in use depends on mode of pathogenesis, geographic distribution, morphology, vector, frequency of schizonts in lymphocytes, frequency of piroplasm stages in erythrocytes, cross immunity and host specificity (Maxie 2016). Pathogenic parasites in *Theileria spp.* include *Theileria parva parva* (East Coast fever), *Theileria parva lawrencei* (Corridor disease), *Theileria parva bovis* (January disease/Rhodesian theileriosis), *Theileria annulata* (Mediterranean or tropical theileriosis) and the *Theileria buffeli* group (*T. buffeli, T. orientalis; T. sergenti*). The parasite reproduces by schizogony in infected lymphocytes, a multiple asexual division (Nene *et al.* 2016). In infected animals the parasite can be found in lymphocytes and in erythrocytes. Pathogenic Theileria spp. causes tick-borne diseases and the primary vectors are the Rhipicephalus and Hyalomma genera.

Turning disease may be caused by *T. parva or T. mutans* (Maxie 2016). It is a sickness that sometimes occurs when partially resistant cattle are exposed to a high infection pressure. Cerebrospinal vessels are occluded by embolisms consisting of parasitized lymphocytes. Hemosiderin staining in a muddy manner in meninges and tissues indicates repetitive minor infarcts. Infarcts of varying ages can be found in other organs such as the kidneys and spleen, these are often small and easy to miss. Koch's bodies can be difficult or impossible to find in lymphoid organs or peripheral blood but are found in abundant amounts in the clumped cells that cause the infarcts.

Corridor disease is caused by *Theileria parva lawrencei*, and it is as well as ECF spread by the *Rhipicephalus* genera (Maxie 2016). The disease resembles ECF clinically and morphologically and there is cross immunity between *T. parva parva* and *T. parva lawrencei*. The parasite is endemic in African buffalo (*Syncerus caffer*). Thus, buffalos may work as carriers.

Tzaneen disease (*Theileria mutans*)

*Theileria mutans* cause Tzaneen disease in cattle (Maxie 2016). It is usually a mild infection, and the disease is widely spread in tropical and subtropical countries. It is as well as the diseases mentioned above spread by ticks of the genera *Rhipicephalus* and some other genera of ticks. Lesions in the lymphoid tissue can be like those caused by ECF but are typically less severe. Reports of fatal disease with haemolytic anaemia, icterus and haemoglobinuria occur but are unusual.

*Theileria annulata* causes the disease tropical theileriosis in the Middle East and central Asia (Maxie 2016). It is spread by ticks from the genus *Hyalomma*. The infection does most commonly not cause severe disease but can occasionally give rise to acute fatal cases with a disease course resembling ECF.

## 2.2 East Coast Fever

East Coast fever is caused by the protozoa *Theileria parva parva* (Nene *et al.* 2016). It is commonly encountered in East and Central Africa or other endemic areas when susceptible animals are exposed to large numbers of infected ticks. The disease remains a problem in several countries, Kenya amongst them where ECF commonly develops into an acute and lethal form. The number of animals developing clinical disease is dependent on the virulence of the infecting strain of T. parva, the number of parasites inoculated and the susceptibility of the host animal (Maxie 2016). Therefore, exposure to T. parva can result in a nonclinical infection but it can also give rise to an acute serious infection with high morbidity and mortality, the mortality can go up to 95%. The main vector for ECF is the tick *Rhipicephalus appendiculatus* (Estrada-Peña & de la Fuente 2014). Macroschizonts can be found in lymphoblasts in the lymph nodes 4 to 20 days after an infected tick has transmitted sporozoites during feeding (Maxie 2016). Approximately 10 days after lymph node infection macroschizonts evolve into the microschizont stage, with this host cell death follows and merozoites ready to invade erythrocytes are released. Approximately 12 days after infection the intraerythrocytic stage of the parasite (piroplasms) starts to appear. Using Giemsa staining the erythrocytes can be round, oval, rod-shaped or anaplasma-like. Typical symptoms of ECF are fever, loss of appetite, enlarged lymph nodes and immune depression. Other symptoms that occur are lacrimation, corneal opacity, nasal discharge and diarrhoea (Gul *et al.* 2015). The incubation period is about 2 weeks and symptoms begin with a high fever followed by red blood cell parasitaemia (Maxie 2016). The clinical picture can express itself and be mistaken for malignant catarrhal fever or other diseases. Another common symptom is pulmonary oedema which can become fatal. Koch's bodies can be found in >60% of lymphocytes in the acute phase of the disease.

During this phase lympholysis and progressive anaemia develop. A typical sign of ECF is leukopenia which is progressive from the onset of fever. The total white blood count often drops below 2 X 10/L and very few leukocytes can be seen in blood smears. The body has a brief onset of myelopoiesis but as the disease progresses neutropenia with immaturity of blood cells develops as well as toxaemia. Hemosiderin accumulates in the macrophages. ECF also causes loss of precursors and depression of the bone marrow which becomes hypoplastic. The cells remaining in the bone marrow are large blastic parasitized lymphocytes and atypical erythroblasts.

The diagnosis is based on clinical signs and diagnostic testing described above. Clinical signs are an important part of the diagnostic procedure, but as there are other diseases, such as malignant catarrhal fever or pneumonia, with similar symptoms the clinical diagnosis may have to be confirmed by e.g. blood sample analysis or post-mortem findings.

### 2.2.1 Autopsy / postmortem findings

The disease gives rise to a general lymphadenopathy (Gul *et al.* 2015). Examining the nodes around the ears are of particular interest since this is *Rhipicephalus appendiculatus'* place of predilection (Maxie 2016). When the lymph nodes are dissected, the cortex has a red-brown color with focal haemorrhages, there is a diffusely spread discoloration and the medullary area is red brown (Zachary 2017). In the acute stage of ECF the spleen is enlarged but it can shrink if the disease has a prolonged course (Maxie 2016). It is common with serous effusion and the connective tissues can have gelatinous or haemorrhagic oedema. In the gastro-intestinal tract ulcerative abomasitis and hemorrhagic enteritis is commonly found (Zachary 2017). In the liver and the kidneys there are proliferative foci of perivascular lymphocytes (Maxie 2016). This can be seen as small grey-white patches which project slightly on the surface of the liver and kidney. The lungs have an increased texture and weight, they become congested and oedematous. In the muscles small haemorrhages occur, these are associated with foci of hyaline degeneration. Petechiae can be seen under the tongue and in the vulva.

### 2.2.2 Histology

Diffuse lymphoid hyperplasia (Gul *et al.* 2015). Lympholysis occurs and is prominent in the germinal centres of the lymph nodes (Maxie 2016). There is a general loss of small lymphocytes, those that remain are large and blastic. Macroschizonts measuring about 2-16 µm in diameter can be found in infected lymphocytes, these are known as Koch's blue bodies and can be used to diagnose ECF. In the liver hepatic periacinar and in some cases also periportal lymphocytic infiltration can be seen. Sometimes there is periacinar hepatocellular necrosis. The bile ducts suffer an irregular canalicular cholestasis and may contain foci of inspissated bile. In the spleen the lymphoid tissue hypertrophies at first, this is later followed by lympholysis. The germinal centres are surrounded by areas of bleeding. Resembling the changes seen in the lymph nodes the hypercellular follicular centres commonly get occupied by hyaline or fibrinous exudate. Capillaries are sometimes blocked by infected leukocytes (Zachary 2017). The lungs change in a characteristic way, they get a lymphocytic infiltration in the interstitial tissue and septum, resulting in a widely spread severe interstitial alveolitis (Maxie 2016). In the kidneys there is infiltration of lymphocytes which is mainly prominent around the vessels and the parietal layer of Bowman's capsule. The kidneys have diffusely scattered multifocal haemorrhage. In the remaining epithelium, areas of parenchymal necrosis with brown pigmentation and hyaline effusions can be seen. The bone marrow becomes hypocellular with asynchrony of the granulocytic system. Lymphocytes proliferate and become abnormally large; they look similar to those that infiltrate other tissues. The erythroid system is less affected.

### 2.2.3 Control methods

To reduce the occurrence of ECF, acaricides, i.e. pesticides used to kill ectoparasites are mainly used (Olwoch *et al.* 2008). The treatment is costly and there are problems with the development of resistance in the tick population. Other problems the treatment faces are animals acting as reservoirs, ineffective monitoring of ECF quarantines, lack of adequate treatment stations, the environmental burden caused by using acaricides and movement of animals to and from treatment stations which causes less growth due to increased energy requirement and decreased grazing time.

### 2.2.4 Lifecycle of *T. Parva*

Understanding the life cycle of the protozoa *T. parva* is important to optimise control strategies (Nene *et al.* 2016). *R. appendiculatus* is a three-host tick and the ellmost important vector for spreading ECF. The disease is spread transstadially. Larvae and nymphs feed on cattle infected with ECF and in doing so they attain erythrocytes containing the piroplasm stage of the parasite. The parasite migrates to the acinar cells in the tick's salivary gland, at this stage the tick is not infectious (Maxie 2016). After onset on feeding, the parasites multiply in the salivary gland and the tick becomes maximally contagious 3-5 days after the onset of feeding. *R. appendiculatus* ticks can get infected and spread the disease from persistently infected cattle when test for parasitaemia is negative (Olds *et al.* 2018).

### 2.2.5 Economy

In addition to economic losses secondary to death, the disease also causes reduced growth, reduced fertility, reduced milk production, paralysis and increases the risk of other secondary parasitic infections (Olwoch *et al.* 2008).

## 2.3 The lifecycle of *Rhipicephalus appendiculatus*

*R. appendiculatus* is a three-host tick species belonging to the genera *Ixode* and the main vector for ECF (Maxie 2016). It feeds on a variety of hosts including cattle and other domestic animals. Exogenous and endogenous factors determine the tick's activity such as host activity, temperature and humidity. The temperate savanna biome is favourable for the ticks, but they are not found in the open savanna (Makwarela *et al.* 2023). There are models used to predict tick abundance and moving in different areas. These models are mainly based on climate data. Factors that are more difficult to include while designing the models are host abundance, resistance, acaricide use and grazing management. (Perry *et al.* 1990). Makwarela *et al.* (2023) writes "Factors such as humidity, temperature, landscape, and rainfall influence the life stages and longevity of ticks". Understanding the geographic distribution of the tick vector is of the utmost importance for the development and implementation of effective means to control tick-borne diseases. It is believed that rainfall increases the transmission of tick-borne diseases such as ECF (Chepkwony *et al.* 2020). When reproducing a high humidity is crucial for the survival of the tick's eggs (Estrada-Peña & de la Fuente 2014). Although the ticks generally thrive in moist and hot conditions, the population is negatively affected by too much moisture and too high temperatures. (Randolph & Rogers 1997).

In one study the moulting behaviour of 4 different tick strains of nymphal *R. appendiculatus* and the involvement of *Theileria* infection in the tick was assessed during different temperatures. The ticks were incubated in constant temperature between 18 and 37 °C while they evolved to their post-moult stage. The ticks developed their highest infection loads in temperatures variating between 23-28 °C. Constant temperatures of 18, 33 and 37 °C were detrimental for development of infection in the salivary gland. (Young & Leitch 1981). The tick population is affected by the availability of host animals and environmental factors. In one study it was concluded that *R. appendiculatus* followed a predictable pattern but that this discovery was of little use since they are highly associated with key resources for the hosts (De Garine-Wichatitsky 2000). This includes permanent waterholes and important forage habitats dominated by Acacia spp. making the ticks unavoidable.

# 3. Aims of the study

The purpose of this study was to investigate whether it is possible to find associations between environmental factors and Boran cattle mortality due to ECF at OPC. This was done by processing data from Boran cattle mortality during the years 2016-2023 and analysing this data together with environmental factors. Since the disease is spread by ticks, factors that could affect these vectors have been selected for the study. Environmental factors analysed are precipitation, biomass, humidity, dew and temperature. A second aim of the study was to verify if the diagnosis of ECF at OPC was correct or mixed up with pneumonia. This was done by analysing the impact of environmental factors in relation to pneumonia as well as ECF to see if the result differed. The study aims to gain a better understanding of how and when the disease is spread by the ticks by identifying environmental risk factors. The long-term goal is to optimise the use of preventative measures.

# 4. Material and Methods

## 4.1 Ol Pejeta Conservancy

Ol Pejeta has the largest herd of purebred Boran cattle in the world and the genetics are valuable to farmers across all of Africa (Ol Pejeta Conservancy n.d.; Butt personal message 2023-10-24). The genetics are passed on to other farmers in the form of bulls, heifers, semen (AI) and embryos. The total herd size at OPC is about 6000 Boran cattle divided into about 5 heards. The cattle graze side by side with the wild animals, including the African buffalo. The integration between the livestock and wildlife makes the use of the grassland more efficient but the integration also raises challenges such as predation and tick-borne diseases. The cattle spend their days grazing the plains of Ol Pejeta and the nights in fenced metal bomas to protect them from predation. The bomas are moved every 3-5 days during the rainy season and every 7-10 days during the dry season. This is done to keep the impact on the land degradation to a minimum. Herders take care of the animals, monitor them and decide where the animals graze. The animals are sprayed with the ectoparasiticide Amitaz which is applied via high-pressure spraying nozzles at special spraying stations to reduce the number of animals falling ill to tick-borne diseases. This is done every seven days during the dry season and every five days during the rainy season. The animals are herded to these stations located in 10 different places over OPC. The last couple of months the cattle have been sprayed with an oil-based solution instead of a water based with the goal to prolong the spraying interval. The beef company’s profit contributes to research, wildlife preservation and salaries amongst other things.

## 4.2 Mortality data

The mortality data used was supplied by OPC and contains daily registrations of cattle mortality and cause of death from 2016.01.01-2023.10.22. The parameters used in this study are the recorded cause of death and the date of death. Since the data included both Boran and Ankole cattle, all registered deaths of Ankole have been excluded from the study. The number of deaths registered as Ankole were a total of 84 during the studies period, none of which deaths were registered as ECF. The remaining data was assumed to be Boran since no other remarks have been made. The mortality recordings have been made daily whether there have been cattle dying or not, but during a total of 168 days no recordings have been made. These days have been excluded from the study since there was no data. In total 2713 days with recordings have been used in the study. The cause of death has been divided into columns, one for each cause. The columns have been filled with the number of deaths each day, days with no death got a 0. When the diagnosis has been uncertain, and several causes have been recorded the number has been split equally between the recorded causes mentioned with a total sum of 1. The mortality data has been used as a cornerstone in the analyses performed.

The diagnosis ECF was made with antemortem findings such as impaired general condition, cough, nasal secretions and inappetence. Criteria for the diagnosis ECF during the postmortem autopsy were generally enlarged lymph nodes, especially around the ears as this is a common place for *R. appendiculatus* to bite. Other findings during the autopsy were commonly inflammation of the airways, especially the trachea and lungs. Petechiae in the oral mucosa and vulva are also common. The autopsy was performed by employees without medical education and these were also the ones placing and reporting the diagnosis, no further diagnostics were made. The antemortem and postmortem findings of ECF can be difficult to distinguish from pneumonia. Pneumonia often presents with cough, fever and inappetence antemortem and give rise to findings such as inflammation of the airways and enlarged lymph nodes postmortem. On several occasions the diagnosis has been noted as ECF/pneumonia when there was uncertainty. The data has then been handled as 0.5 cattle dying from ECF and 0.5 from pneumonia. The data from ECF and pneumonia were ranked where each day was given a number from 0-2 where 0 = no death, 1 = 0.5-1.0 deaths and 2 = >1.0 deaths. This ranking is later referred to as ECF012 and Pnemonia012. During the investigated period a total of 262,5 deaths were registered as ECF spread out over 221 days and 105,5 as pneumonia spread out over 104 days.

## 4.3 Weather data

Weather data from 2015.11.01-2023.10.22 was obtained from the weather database Visual Crossing (Visual Crossing n.d.). Daily recordings of the weather data have been used in the study and compared with the mortality data. To be able to see the connection between ECF and weather back in time, a time lag for the weather data has been used. This has been done by averaging the weather data from the current date and six days back, this value has then been advanced by 7, 14, 21, 28, 35 and 42 days. To obtain values from the beginning of the mortality data from 2016-01-

01, weather data from December and November 2015 have been used. Before analysing the 168 dates missing from the mortality data were removed from the weather data

Precipitation data was obtained from OPC and contained daily recordings from ten weather stations within OPC. A mean of these stations has been used and processed in the same way as the weather data from Visual Crossing. Furthermore, the mean of the last 7, 30, 60 and 90 days were also examined as a way of estimating the biomass.

## 4.4 Biomass (Grass height)

Methods for measuring access to grass as food for our livestock have been developed as there is a need to optimise production from an economic point of view but also from an environmental perspective (Ali et al. 2016). In this study the biomass data used is based on satellite images taken over OPC once a week during the period 2017-02-18 to 2023-10-10. The index used was MSAVI2. Days with clouds have been excluded as these do not provide any useful data. The biomass is based on 10 areas within OPC with open grassland, the average value of these points has been calculated day by day. As the data is collected once a week and cloudiness sometimes cause longer measurement intervals, the daily biomass has been calculated by assuming the value changes linearly between measurement points. The formula (x2-x1)/(n+1) has been used to calculate the average daily change where x=biomass and n=number of days between the measurements. The average daily change has been added with the previous day's value to fill in the days where data was missing.

## 4.5 Analyses

All analyses have been made in the statistics program Minitab (Data Analysis, Statistical & Process Improvement Tools |[Minitab n.d.]). Descriptive diagnostics were made with the environmental factors as variables and the by variables being the ranked ECF and pneumonia mortality data. Environmental factors examined were daily registrations of precipitation (mm) from OPC, weather parameters from Vissual Crossing were mean temperature (°C), minimum temperature (°C), maximum temperature (°C), humidity (%) and dew (°C) (dewpoint). Biomass data from satellites was also analysed. Using descriptive diagnostics, the ECF class, total count of days, the mean value from the environmental factors, the standard error of the mean, the minimum and the maximum values was obtained. The P-value was calculated by Anova Fit General Linear Model with the environmental factors as responses and the classed mortality data as factors. The Anova General Linear

Model Comparisons was used to examine whether these were significant differences between the different classes of mortality data. Type of pairwise comparisons, was calculated thit the Tukey method with environmental factors as response and the classed mortality data as term for comparison. Means that do not share a letter were significantly different.

# 5. Results

## 5.1 ECF

The following tables and figures show the effect of environmental factors on deaths caused by ECF.

### 5.1.1 Humidity

*Table 1. Humidity data where the orange column shows the period examined. The blue columns show the results from the descriptive diagnostics. Rank: 0 = days with no death due to ECF, 1 = days with 0.5-1.0 dead cattle due to ECF and 2 = days with >1 dead cattle due to ECF. Total Count = the number of days each rank occurred. Mean = the mean value of the humidity data from the examined period prior to the date analysed. This has been calculated by summarising the daily humidity data from the examined week and dividing it by 7. The green part of the table shows the P-value; the yellow part if there is any overlap between the different rankings. Groupings that do not share a letter are significantly different.*

| Variable | ECF012 | Total Count | Mean | SE Mean | Minimum | Maximum | P-Value | ECF012 | Grouping: | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Rank | | (%) | (%) | (%) | (%) | | Rank | A | B | C |
| **Mean Humidity 0-6 Days Prior** | 0 | 2492 | 77,38 | 0,15 | 53,67 | 90,50 | 0,006 | 2 | A | | |
| | 1 | 188 | 78,59 | 0,51 | 57,13 | 88,86 | | 1 | A | B | |
| | 2 | 33 | 80,35 | 1,11 | 62,89 | 87,54 | | 0 | | B | |
| | | | | | | | | | | | |
| **Mean Humidity 7-13 Days Prior** | 0 | 2492 | 77,36 | 0,15 | 53,67 | 90,50 | 0,000 | 2 | A | | |
| | 1 | 188 | 78,91 | 0,49 | 58,27 | 90,06 | | 1 | A | | |
| | 2 | 33 | 81,44 | 1,03 | 64,61 | 88,03 | | 0 | | B | |
| | | | | | | | | | | | |
| **Mean Humidity 14-20 Days Prior** | 0 | 2492 | 77,33 | 0,15 | 53,67 | 90,50 | 0,000 | 2 | A | | |
| | 1 | 188 | 79,02 | 0,48 | 57,84 | 89,87 | | 1 | A | | |
| | 2 | 33 | 81,29 | 1,22 | 59,06 | 89,11 | | 0 | | B | |
| | | | | | | | | | | | |
| **Mean Humidity 21-27 Days Prior** | 0 | 2492 | 77,26 | 0,14 | 53,67 | 90,50 | 0,000 | 2 | A | | |
| | 1 | 188 | 79,59 | 0,50 | 60,30 | 89,21 | | 1 | A | | |
| | 2 | 33 | 80,82 | 1,14 | 61,64 | 88,27 | | 0 | | B | |
| | | | | | | | | | | | |
| **Mean Humidity 28-34 Days Prior** | 0 | 2492 | 77,36 | 0,15 | 53,67 | 90,29 | 0,000 | 2 | A | | |
| | 1 | 188 | 79,35 | 0,49 | 57,33 | 90,50 | | 1 | A | | |
| | 2 | 33 | 81,33 | 1,05 | 63,21 | 87,73 | | 0 | | B | |
| | | | | | | | | | | | |
| **Mean Humidity 35-41 Days Prior** | 0 | 2492 | 77,51 | 0,15 | 53,67 | 90,50 | 0,000 | 2 | A | | |
| | 1 | 188 | 79,32 | 0,47 | 57,90 | 89,26 | | 1 | A | | |
| | 2 | 33 | 80,53 | 0,98 | 60,71 | 87,51 | | 0 | | B | |
| | | | | | | | | | | | |
| **Mean Humidity 42-48 Days Prior** | 0 | 2492 | 77,63 | 0,15 | 53,67 | 90,50 | 0,004 | 1 | A | | |
| | 1 | 188 | 79,32 | 0,47 | 57,40 | 89,50 | | 2 | A | B | |
| | 2 | 33 | 79,30 | 1,01 | 63,11 | 87,14 | | 0 | | B | |

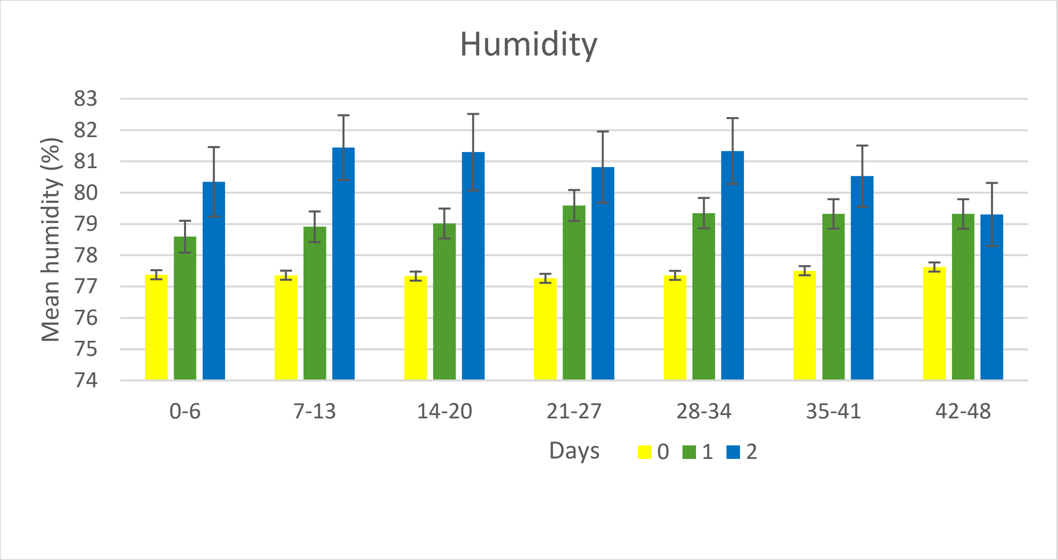
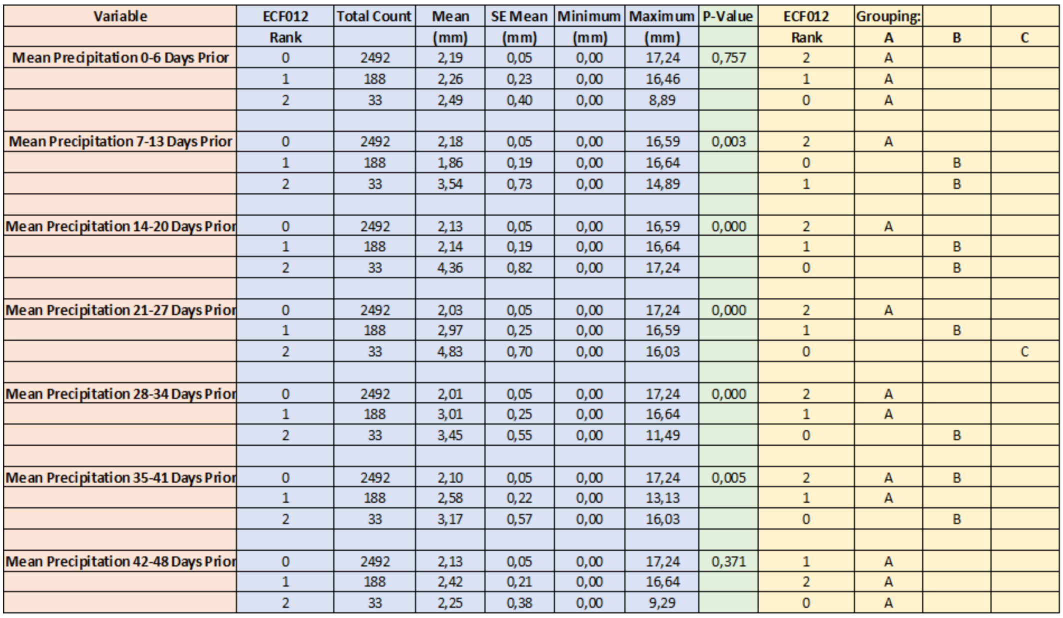


*Figure 1. On the Y-axis the daily mean humidity is displayed as a percentage and the X-axis shows the period examined prior to the analysed date. Yellow bars = rank 0, days with no death due to ECF, green bars = rank 1 with 0.5-1.0 dead cattle due to ECF, blue bars = rank 2 with >1 dead cattle due to ECF. The black lines on the bars illustrate the standard error of the mean.*

Table 1 show that no cattle died in ECF during 2492 of the examined dates during the studies period 2016.01.01-2023.10.22. On 188 of the days 0.5-1.0 cattle died and on 33 of the days >1.0 cattle died. The results from the analyses of humidity in relation to mortality due to ECF illustrated in table 1 and figure 1 show a relationship where the values for humidity are higher in rankings 1 and/or 2 than in 0, with p <0.01 for all time lags.

## 5.1.2 Precipitation

*Table 2. Precipitation data where the orange column shows the period examined. The blue columns show the results from the descriptive diagnostics. Rank: 0 = days with no death due to ECF, 1 = days with 0.5-1.0 dead cattle due to ECF and 2 = days with >1 dead cattle due to ECF. Total Count = the number of days each rank occurred. Mean = the mean value of the precipitation data from the examined period prior to the date analysed. This has been calculated by summarising the daily humidity data from the examined week and dividing it by 7. The green part of the table shows the P-value; the yellow part if there is any overlap between the different rankings. Groupings that do not share a letter are significantly different.*



| Variable | ECF012 Rank | Total Count | Mean (mm) | SE Mean (mm) | Minimum (mm) | Maximum (mm) | P-Value | ECF012 Rank | Grouping: | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | A | B | C |
| **Mean Precipitation 0-6 Days Prior** | 0 | 2492 | 2,19 | 0,05 | 0,00 | 17,24 | 0,757 | 2 | A | | |
| | 1 | 188 | 2,26 | 0,23 | 0,00 | 16,46 | | 1 | A | | |
| | 2 | 33 | 2,49 | 0,40 | 0,00 | 8,89 | | 0 | A | | |
| | | | | | | | | | | | |
| **Mean Precipitation 7-13 Days Prior** | 0 | 2492 | 2,18 | 0,05 | 0,00 | 16,59 | 0,003 | 2 | A | | |
| | 1 | 188 | 1,86 | 0,19 | 0,00 | 16,64 | | 0 | | B | |
| | 2 | 33 | 3,54 | 0,73 | 0,00 | 14,89 | | 1 | | B | |
| | | | | | | | | | | | |
| **Mean Precipitation 14-20 Days Prior** | 0 | 2492 | 2,13 | 0,05 | 0,00 | 16,59 | 0,000 | 2 | A | | |
| | 1 | 188 | 2,14 | 0,19 | 0,00 | 16,64 | | 1 | | B | |
| | 2 | 33 | 4,36 | 0,82 | 0,00 | 17,24 | | 0 | | B | |
| | | | | | | | | | | | |
| **Mean Precipitation 21-27 Days Prior** | 0 | 2492 | 2,03 | 0,05 | 0,00 | 17,24 | 0,000 | 2 | A | | |
| | 1 | 188 | 2,97 | 0,25 | 0,00 | 16,59 | | 1 | | B | |
| | 2 | 33 | 4,83 | 0,70 | 0,00 | 16,03 | | 0 | | | C |
| | | | | | | | | | | | |
| **Mean Precipitation 28-34 Days Prior** | 0 | 2492 | 2,01 | 0,05 | 0,00 | 17,24 | 0,000 | 2 | A | | |
| | 1 | 188 | 3,01 | 0,25 | 0,00 | 16,64 | | 1 | A | | |
| | 2 | 33 | 3,45 | 0,55 | 0,00 | 11,49 | | 0 | | B | |
| | | | | | | | | | | | |
| **Mean Precipitation 35-41 Days Prior** | 0 | 2492 | 2,10 | 0,05 | 0,00 | 17,24 | 0,005 | 2 | A | B | |
| | 1 | 188 | 2,58 | 0,22 | 0,00 | 13,13 | | 1 | A | | |
| | 2 | 33 | 3,17 | 0,57 | 0,00 | 16,03 | | 0 | | B | |
| | | | | | | | | | | | |
| **Mean Precipitation 42-48 Days Prior** | 0 | 2492 | 2,13 | 0,05 | 0,00 | 17,24 | 0,371 | 1 | A | | |
| | 1 | 188 | 2,42 | 0,21 | 0,00 | 16,64 | | 2 | A | | |
| | 2 | 33 | 2,25 | 0,38 | 0,00 | 9,29 | | 0 | A | | |

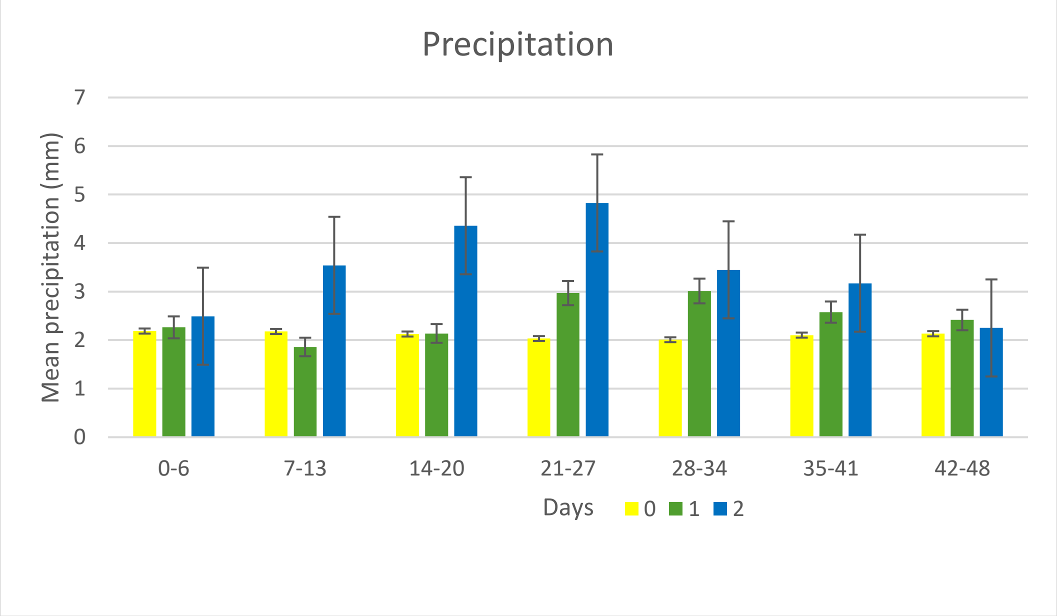
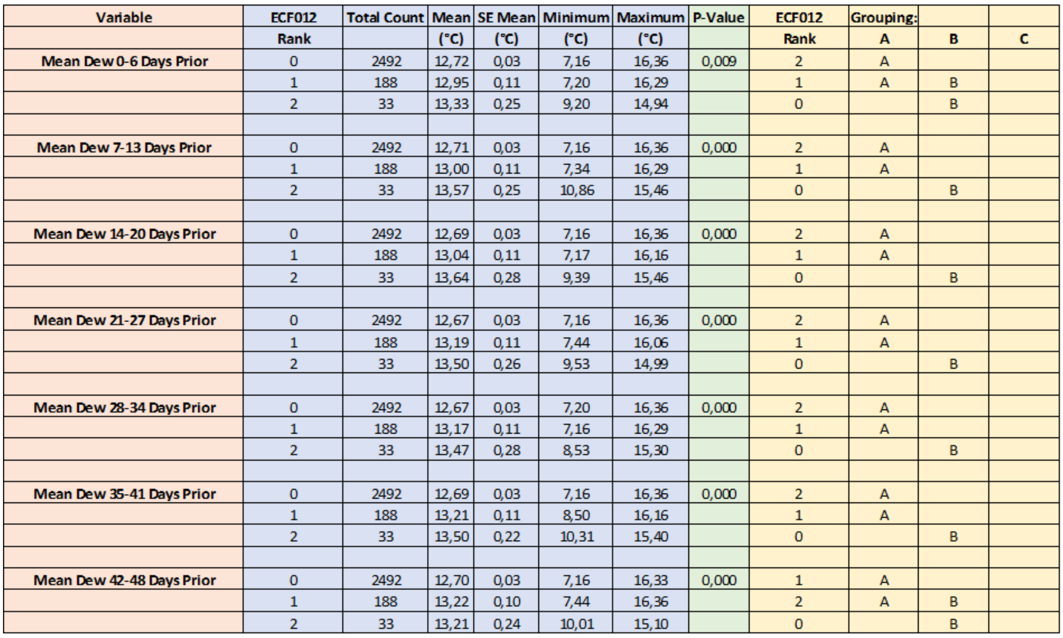


*Figure 2. On the Y-axis the daily mean precipitation is displayed i mm and the X-axis shows the period examined prior to the analysed date. Yellow bars = rank 0, days with no death due to ECF, green bars = rank 1 with 0.5-1.0 dead cattle due to ECF, blue bars = rank 2 with >1 dead cattle due to ECF. The black lines on the bars illustrate the standard error of the mean.*

Generally table 2 and figure 2 shows a trend with more precipitation prior to death due to ECF, this trend is most prominent 21-27 days prior the examined date where the mean precipitation difference is big between the different rankings. Between 7 and 41 days prior the cattle's death, there was is a significant difference between the rankings ($p < 0.01$). No statistically significant findings were found 0-6 and 42-48 days prior to cattle dying from ECF.

### 5.1.3 Dew

*Table 3. Dew data where the orange column shows the period examined. The blue columns show the results from the descriptive diagnostics. Rank: 0 = days with no death due to ECF, 1 = days with 0.5-1.0 dead cattle due to ECF and 2 = days with >1 dead cattle due to ECF. Total Count = the number of days each rank occurred. Mean = the mean value of the dew data from the examined period prior to the date analysed. This has been calculated by summarising the daily humidity data from the examined week and dividing it by 7. The green part of the table shows the P-value; the yellow part if there is any overlap between the different rankings. Groupings that do not share a letter are significantly different.*



| Variable | ECF012 | Total Count | Mean | SE Mean | Minimum | Maximum | P-Value | ECF012 | Grouping: | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Rank | | (°C) | (°C) | (°C) | (°C) | | Rank | A | B | C |
| **Mean Dew 0-6 Days Prior** | 0 | 2492 | 12,72 | 0,03 | 7,16 | 16,36 | 0,009 | 2 | A | | |
| | 1 | 188 | 12,95 | 0,11 | 7,20 | 16,29 | | 1 | A | B | |
| | 2 | 33 | 13,33 | 0,25 | 9,20 | 14,94 | | 0 | | B | |
| | | | | | | | | | | | |
| **Mean Dew 7-13 Days Prior** | 0 | 2492 | 12,71 | 0,03 | 7,16 | 16,36 | 0,000 | 2 | A | | |
| | 1 | 188 | 13,00 | 0,11 | 7,34 | 16,29 | | 1 | A | | |
| | 2 | 33 | 13,57 | 0,25 | 10,86 | 15,46 | | 0 | | B | |
| | | | | | | | | | | | |
| **Mean Dew 14-20 Days Prior** | 0 | 2492 | 12,69 | 0,03 | 7,16 | 16,36 | 0,000 | 2 | A | | |
| | 1 | 188 | 13,04 | 0,11 | 7,17 | 16,16 | | 1 | A | | |
| | 2 | 33 | 13,64 | 0,28 | 9,39 | 15,46 | | 0 | | B | |
| | | | | | | | | | | | |
| **Mean Dew 21-27 Days Prior** | 0 | 2492 | 12,67 | 0,03 | 7,16 | 16,36 | 0,000 | 2 | A | | |
| | 1 | 188 | 13,19 | 0,11 | 7,44 | 16,06 | | 1 | A | | |
| | 2 | 33 | 13,50 | 0,26 | 9,53 | 14,99 | | 0 | | B | |
| | | | | | | | | | | | |
| **Mean Dew 28-34 Days Prior** | 0 | 2492 | 12,67 | 0,03 | 7,20 | 16,36 | 0,000 | 2 | A | | |
| | 1 | 188 | 13,17 | 0,11 | 7,16 | 16,29 | | 1 | A | | |
| | 2 | 33 | 13,47 | 0,28 | 8,53 | 15,30 | | 0 | | B | |
| | | | | | | | | | | | |
| **Mean Dew 35-41 Days Prior** | 0 | 2492 | 12,69 | 0,03 | 7,16 | 16,36 | 0,000 | 2 | A | | |
| | 1 | 188 | 13,21 | 0,11 | 8,50 | 16,16 | | 1 | A | | |
| | 2 | 33 | 13,50 | 0,22 | 10,31 | 15,40 | | 0 | | B | |
| | | | | | | | | | | | |
| **Mean Dew 42-48 Days Prior** | 0 | 2492 | 12,70 | 0,03 | 7,16 | 16,33 | 0,000 | 1 | A | | |
| | 1 | 188 | 13,22 | 0,10 | 7,44 | 16,36 | | 2 | A | B | |
| | 2 | 33 | 13,21 | 0,24 | 10,01 | 15,10 | | 0 | | B | |

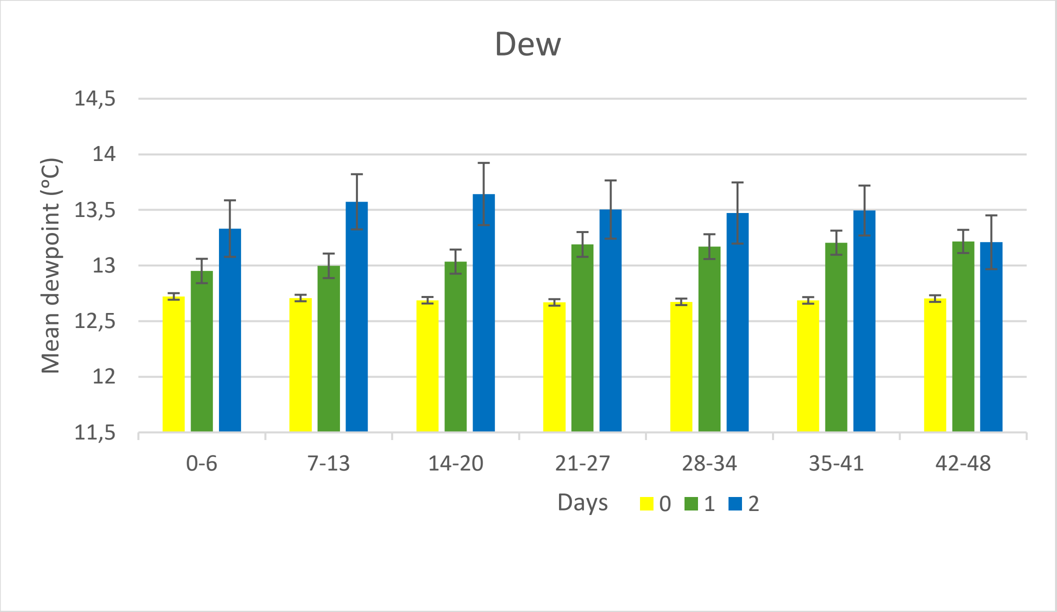


*Figure 3. On the Y-axis the daily mean dew (dewpoint) is displayed in °C and the X-axis shows the period examined prior to the analysed date. Yellow bars = rank 0, days with no death due to ECF, green bars = rank 1 with 0.5-1.0 dead cattle due to ECF, blue bars = rank 2 with >1 dead cattle due to ECF. The black lines on the bars illustrate the standard error of the mean.*

The results from the analyses of dew in relation to mortality in ECF illustrated in table 3 and figure 3 show a connection where the values for dew are higher in rankings 1 & 2 than in 0. Some overlap exists between the different rankings 0-2. There are no major differences between the different time lags analysed.

### 5.1.3 Biomass

*Table 4. Biomass data where the orange column shows the period examined. The blue columns show the results from the descriptive diagnostics. Rank: 0 = days with no death due to ECF, 1 = days with 0.5-1.0 dead cattle due to ECF and 2 = days with >1 dead cattle due to ECF. Total Count = the number of days each rank occurred. Mean = the mean value of the biomass data from the examined period prior to the date analysed. This has been calculated by summarising the daily humidity data from the examined week and dividing it by 7. The green part of the table shows the P-value; the yellow part if there is any overlap between the different rankings. Groupings that do not share a letter are significantly different.*

| Variable | ECF012 | Total Count | Mean | SE Mean | Minimum | Maximum | P-Value | ECF012 | Grouping: | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Rank | | (MSAVI2) | (MSAVI2) | (MSAVI2) | (MSAVI2) | | Rank | A | B | C |
| **Mean Biomass 0-6 Days Prior** | 0 | 2084 | 0,46 | 0,00 | 0,23 | 0,81 | 0,000 | 2 | A | | |
| | 1 | 159 | 0,51 | 0,01 | 0,23 | 0,81 | | 1 | | B | |
| | 2 | 28 | 0,58 | 0,03 | 0,25 | 0,75 | | 0 | | | C |
| | | | | | | | | | | | |
| **Mean Biomass 7-13 Days Prior** | 0 | 2084 | 0,46 | 0,00 | 0,23 | 0,81 | 0,000 | 2 | A | | |
| | 1 | 159 | 0,51 | 0,01 | 0,24 | 0,80 | | 1 | A | | |
| | 2 | 28 | 0,58 | 0,03 | 0,26 | 0,75 | | 0 | | B | |
| | | | | | | | | | | | |
| **Mean Biomass 14-20 Days Prior** | 0 | 2084 | 0,46 | 0,00 | 0,23 | 0,81 | 0,000 | 2 | A | | |
| | 1 | 159 | 0,51 | 0,01 | 0,25 | 0,81 | | 1 | A | | |
| | 2 | 28 | 0,57 | 0,03 | 0,25 | 0,75 | | 0 | | B | |
| | | | | | | | | | | | |
| **Mean Biomass 21-27 Days Prior** | 0 | 2084 | 0,46 | 0,00 | 0,23 | 0,81 | 0,000 | 2 | A | | |
| | 1 | 159 | 0,51 | 0,01 | 0,24 | 0,81 | | 1 | A | | |
| | 2 | 28 | 0,56 | 0,03 | 0,27 | 0,75 | | 0 | | B | |
| | | | | | | | | | | | |
| **Mean Biomass 28-34 Days Prior** | 0 | 2084 | 0,46 | 0,00 | 0,23 | 0,81 | 0,000 | 2 | A | | |
| | 1 | 159 | 0,50 | 0,01 | 0,25 | 0,80 | | 1 | A | | |
| | 2 | 28 | 0,54 | 0,03 | 0,28 | 0,74 | | 0 | | B | |
| | | | | | | | | | | | |
| **Mean Biomass 35-41 Days Prior** | 0 | 2084 | 0,46 | 0,00 | 0,23 | 0,81 | 0,001 | 2 | A | | |
| | 1 | 159 | 0,50 | 0,01 | 0,25 | 0,81 | | 1 | A | | |
| | 2 | 28 | 0,52 | 0,03 | 0,29 | 0,73 | | 0 | | B | |
| | | | | | | | | | | | |
| **Mean Biomass 42-48 Days Prior** | 0 | 2084 | 0,46 | 0,00 | 0,23 | 0,81 | 0,021 | 2 | A | B | |
| | 1 | 159 | 0,49 | 0,01 | 0,25 | 0,77 | | 1 | A | | |
| | 2 | 28 | 0,50 | 0,02 | 0,31 | 0,72 | | 0 | | B | |

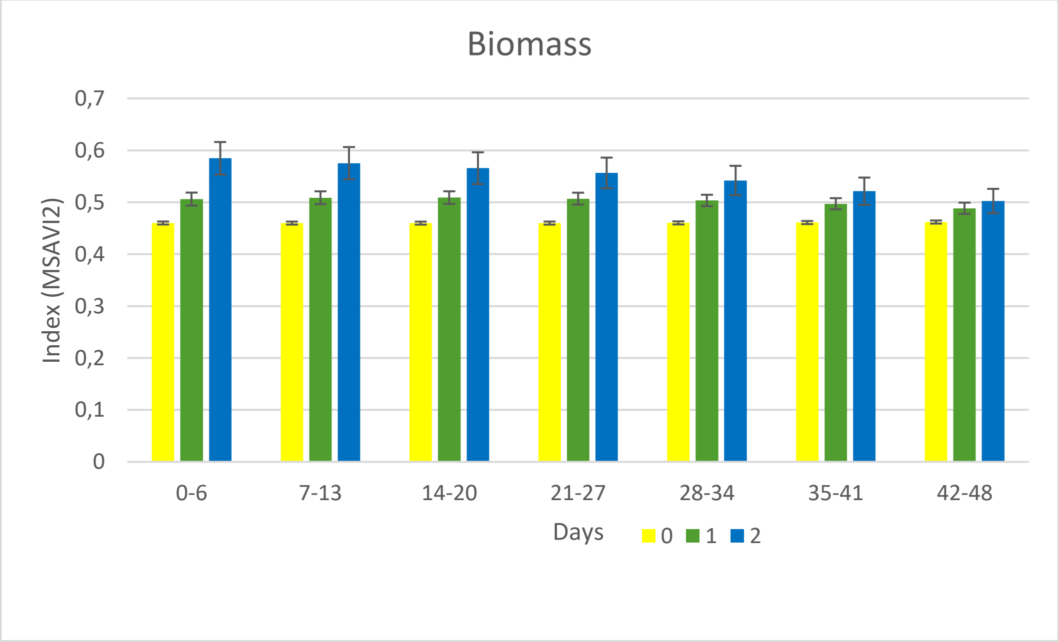


*Figure 4. On the Y-axis the daily mean biomass is displayed as an index (MSAVI2) and the X-axis shows the period examined prior to the analysed date. Yellow bars = rank 0, days with no death due to ECF, green bars = rank 1 with 0.5-1.0 dead cattle due to ECF, blue bars = rank 2 with >1 dead cattle due to ECF. The black lines on the bars illustrate the standard error of the mean.*

Table 4 and figure 4 show a significant effect of higher biomass on mortality due to ECF in all times laps (P<0.03).

*Table 5. The daily mean precipitation data has been examined from the analysed date and some days back in time illustrated by the variables in the orange column. The precipitation data was in these analyses used as an indirect measurement for the biomass and grass height. The blue columns show the results from the descriptive diagnostics. Rank: 0 = days with no death due to ECF, 1 = days with 0.5-1.0 dead cattle due to ECF and 2 = days with >1 dead cattle due to ECF. Total Count = the number of days each rank occurred. Mean = the mean value of the biomass data from the examined period prior to the date analysed. This has been calculated by summarising the daily humidity data from the examined week and dividing it by 7. The green part of the table shows the P-value; the yellow part if there is any overlap between the different rankings. Groupings that do not share a letter are significantly different.*

| Variable | ECF012 | Total Count | Mean | SE Mean | Minimum | Maximum | P-Value | ECF012 | Grouping | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Rank | | (mm) | (mm) | (mm) | (mm) | | Rank | A | B | C |
| Precipitation Mean 7 days | 0 | 2492 | 2,20 | 0,05 | 0,00 | 17,24 | 0,800 | 2 | A | | |
| | 1 | 188 | 2,26 | 0,23 | 0,00 | 16,46 | | 1 | A | | |
| | 2 | 33 | 2,49 | 0,40 | 0,00 | 8,89 | | 0 | A | | |
| | | | | | | | | | | | |
| Precipitation Mean 30 days | 0 | 2492 | 2,11 | 0,03 | 0,00 | 8,38 | 0,000 | 2 | A | | |
| | 1 | 188 | 2,39 | 0,13 | 0,00 | 8,38 | | 1 | | B | |
| | 2 | 33 | 3,90 | 0,47 | 0,49 | 8,38 | | 0 | | B | |
| | | | | | | | | | | | |
| Precipitation Mean 60 days | 0 | 2492 | 2,08 | 0,02 | 0,00 | 7,00 | 0,000 | 2 | A | | |
| | 1 | 188 | 2,45 | 0,11 | 0,10 | 6,30 | | 1 | | B | |
| | 2 | 33 | 3,32 | 0,33 | 0,30 | 6,40 | | 0 | | | C |
| | | | | | | | | | | | |
| Precipitation Mean 90 days | 0 | 2492 | 2,15 | 0,02 | 0,46 | 5,42 | 0,000 | 2 | A | | |
| | 1 | 188 | 2,62 | 0,09 | 0,53 | 5,40 | | 1 | | B | |
| | 2 | 33 | 3,19 | 0,28 | 0,50 | 5,43 | | 0 | | | C |

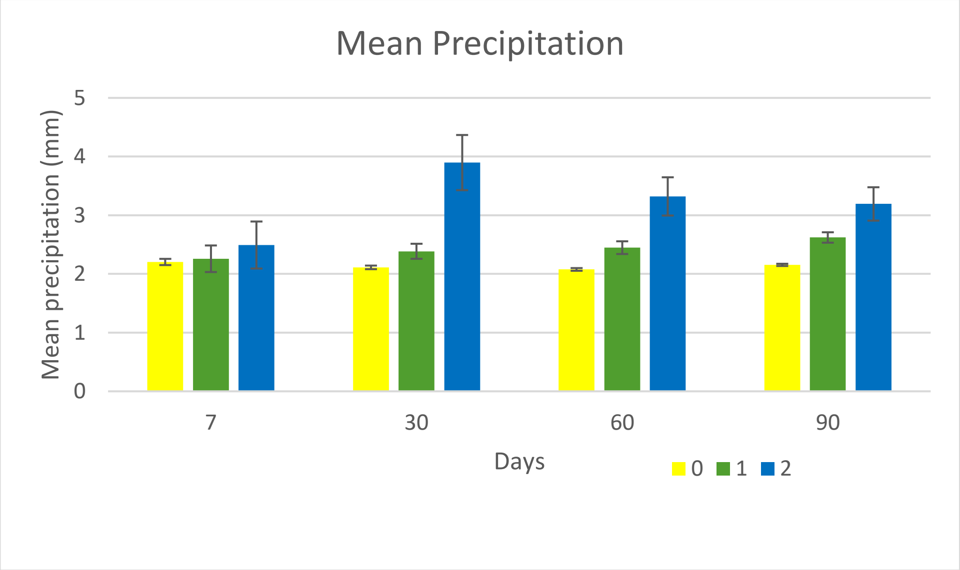


*Figure 5. On the Y-axis the daily mean precipitation is displayed i mm and the X-axis shows the period examined prior to the analysed date. Yellow bars = rank 0, days with no death due to ECF, green bars = rank 1 with 0.5-1.0 dead cattle due to ECF, blue bars = rank 2 with >1 dead cattle due to ECF. The black lines on the bars illustrate the standard error of the mean.*

Table 5 and figure 5 show that no significant connection was found between ECF mortality and last week's average precipitation. Statistically significant findings were found when analysing the mean of the last 30, 60 and 90 days (P<0.001). The analyses show higher values of precipitation before cattle die. When examining the relationship 30 days before death, rankings 0 & 1 have a lot of overlap between the groups. The average precipitation over the last 60 or 90 days shows the strongest relationship and has significant differences between all three different rankings. However, this relationship is strongest for the average precipitation in the last 60 days with the strongest differences between the mean value and the lowest standard error of the mean compared to the other variables.

### 5.1.4 Temperature

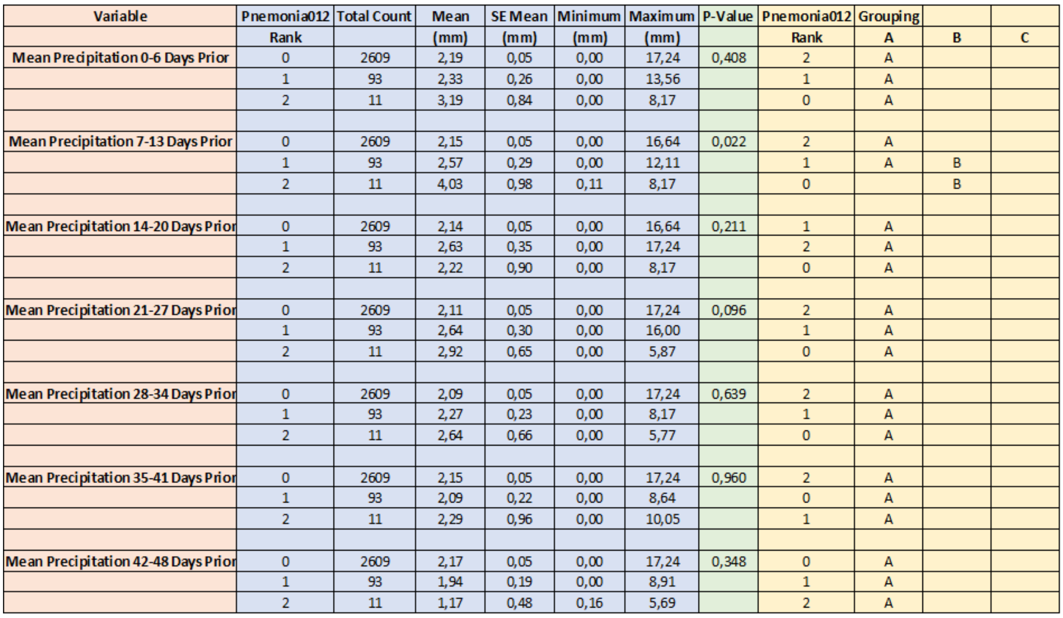
When analysing the daily minimum, mean and maximum temperature no relation to death due to ECF was found.

## 5.2 Pneumonia

The following tables and figures show the effect of environmental factors on deaths caused by precipitation.

### 5.2.1 Precipitation

*Table 6. Precipitation data where the orange column shows the period examined. The blue columns show the results from the descriptive diagnostics. Rank: 0 = days with no death due to pneumonia, 1 = days with 0.5-1.0 dead cattle due to pneumonia and 2 = days with >1 dead cattle due to pneumonia. Total Count = the number of days each rank occurred. Mean = the mean value of the precipitation data from the examined period prior to the date analysed. This has been calculated by summarising the daily humidity data from the examined week and dividing it by 7. The green part of the table shows the P-value; the yellow part if there is any overlap between the different rankings. Groupings that do not share a letter are significantly different.*



| Variable | Pnemonia012 | Total Count | Mean | SE Mean | Minimum | Maximum | P-Value | Pnemonia012 | Grouping | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Rank | | (mm) | (mm) | (mm) | (mm) | | Rank | A | B | C |
| **Mean Precipitation 0-6 Days Prior** | 0 | 2609 | 2,19 | 0,05 | 0,00 | 17,24 | 0,408 | 2 | A | | |
| | 1 | 93 | 2,33 | 0,26 | 0,00 | 13,56 | | 1 | A | | |
| | 2 | 11 | 3,19 | 0,84 | 0,00 | 8,17 | | 0 | A | | |
| | | | | | | | | | | | |
| **Mean Precipitation 7-13 Days Prior** | 0 | 2609 | 2,15 | 0,05 | 0,00 | 16,64 | 0,022 | 2 | A | | |
| | 1 | 93 | 2,57 | 0,29 | 0,00 | 12,11 | | 1 | A | B | |
| | 2 | 11 | 4,03 | 0,98 | 0,11 | 8,17 | | 0 | | B | |
| | | | | | | | | | | | |
| **Mean Precipitation 14-20 Days Prior** | 0 | 2609 | 2,14 | 0,05 | 0,00 | 16,64 | 0,211 | 1 | A | | |
| | 1 | 93 | 2,63 | 0,35 | 0,00 | 17,24 | | 2 | A | | |
| | 2 | 11 | 2,22 | 0,90 | 0,00 | 8,17 | | 0 | A | | |
| | | | | | | | | | | | |
| **Mean Precipitation 21-27 Days Prior** | 0 | 2609 | 2,11 | 0,05 | 0,00 | 17,24 | 0,096 | 2 | A | | |
| | 1 | 93 | 2,64 | 0,30 | 0,00 | 16,00 | | 1 | A | | |
| | 2 | 11 | 2,92 | 0,65 | 0,00 | 5,87 | | 0 | A | | |
| | | | | | | | | | | | |
| **Mean Precipitation 28-34 Days Prior** | 0 | 2609 | 2,09 | 0,05 | 0,00 | 17,24 | 0,639 | 2 | A | | |
| | 1 | 93 | 2,27 | 0,23 | 0,00 | 8,17 | | 1 | A | | |
| | 2 | 11 | 2,64 | 0,66 | 0,00 | 5,77 | | 0 | A | | |
| | | | | | | | | | | | |
| **Mean Precipitation 35-41 Days Prior** | 0 | 2609 | 2,15 | 0,05 | 0,00 | 17,24 | 0,960 | 2 | A | | |
| | 1 | 93 | 2,09 | 0,22 | 0,00 | 8,64 | | 0 | A | | |
| | 2 | 11 | 2,29 | 0,96 | 0,00 | 10,05 | | 1 | A | | |
| | | | | | | | | | | | |
| **Mean Precipitation 42-48 Days Prior** | 0 | 2609 | 2,17 | 0,05 | 0,00 | 17,24 | 0,348 | 0 | A | | |
| | 1 | 93 | 1,94 | 0,19 | 0,00 | 8,91 | | 1 | A | | |
| | 2 | 11 | 1,17 | 0,48 | 0,16 | 5,69 | | 2 | A | | |

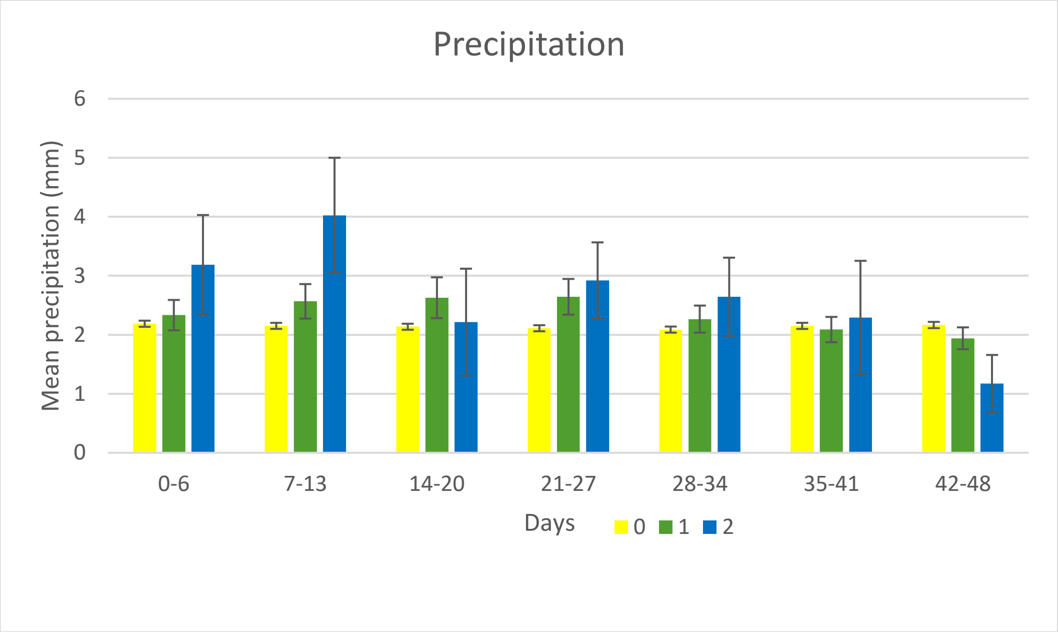


*Figure 6. On the Y-axis the daily mean precipitation is displayed in mm and the X-axis shows the period examined prior to the analysed date. Yellow bars = rank 0, days with no death due to pneumonia, green bars = rank 1 with 0.5-1.0 dead cattle due to pneumonia, blue bars = rank 2 with >1 dead cattle due to pneumonia. The black lines on the bars illustrate the standard error of the mean.*

7-13 days prior to death due to pneumonia table 6 and figure 6 show a significant rise in the amount of rainfall when cattle die compared to when no cattle die due to pneumonia.

## 5.2.2 Biomass

*Table 7. Biomass data where the orange column shows the period examined. The blue columns show the results from the descriptive diagnostics. Rank: 0 = days with no death due to pneumonia, 1 = days with 0.5-1.0 dead cattle due to pneumonia and 2 = days with >1 dead cattle due to pneumonia. Total Count = the number of days each rank occurred. Mean = the mean value of the biomass data from the examined period prior to the date analysed. This has been calculated by summarising the daily humidity data from the examined week and dividing it by 7. The green part of the table shows the P-value; the yellow part if there is any overlap between the different rankings. Groupings that do not share a letter are significantly different.*

| Variable | Pnemonia012 | Total Count | Mean | SE Mean | Minimum | Maximum | P-Value | Pnemonia012 | Grouping: | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Rank | | (MSAVI2) | (MSAVI2) | (MSAVI2) | (MSAVI2) | | Rank | A | B | C |
| **Mean Biomass 0-6 Days Prior** | 0 | 2234 | 0,46 | 0,00 | 0,23 | 0,81 | 0,887 | 2 | A | | |
| | 1 | 75 | 0,46 | 0,02 | 0,23 | 0,79 | | 1 | A | | |
| | 2 | 10 | 0,48 | 0,02 | 0,37 | 0,52 | | 0 | A | | |
| | | | | | | | | | | | |
| **Mean Biomass 7-13 Days Prior** | 0 | 2234 | 0,46 | 0,00 | 0,23 | 0,81 | 0,916 | 0 | A | | |
| | 1 | 75 | 0,46 | 0,02 | 0,24 | 0,81 | | 1 | A | | |
| | 2 | 10 | 0,45 | 0,02 | 0,37 | 0,51 | | 2 | A | | |
| | | | | | | | | | | | |
| **Mean Biomass 14-20 Days Prior** | 0 | 2234 | 0,46 | 0,00 | 0,23 | 0,81 | 0,238 | 0 | A | | |
| | 1 | 75 | 0,45 | 0,02 | 0,24 | 0,80 | | 1 | A | | |
| | 2 | 10 | 0,39 | 0,02 | 0,31 | 0,47 | | 2 | A | | |
| | | | | | | | | | | | |
| **Mean Biomass 21-27 Days Prior** | 0 | 2234 | 0,46 | 0,00 | 0,23 | 0,81 | 0,009 | 0 | A | | |
| | 1 | 75 | 0,44 | 0,01 | 0,24 | 0,79 | | 1 | A | B | |
| | 2 | 10 | 0,34 | 0,02 | 0,26 | 0,40 | | 2 | | B | |
| | | | | | | | | | | | |
| **Mean Biomass 28-34 Days Prior** | 0 | 2234 | 0,46 | 0,00 | 0,23 | 0,81 | 0,000 | 0 | A | | |
| | 1 | 75 | 0,44 | 0,01 | 0,23 | 0,80 | | 1 | A | | |
| | 2 | 10 | 0,30 | 0,02 | 0,25 | 0,40 | | 2 | | B | |
| | | | | | | | | | | | |
| **Mean Biomass 35-41 Days Prior** | 0 | 2234 | 0,47 | 0,00 | 0,23 | 0,81 | 0,000 | 0 | A | | |
| | 1 | 75 | 0,43 | 0,02 | 0,24 | 0,81 | | 1 | A | | |
| | 2 | 10 | 0,29 | 0,02 | 0,25 | 0,43 | | 2 | | B | |
| | | | | | | | | | | | |
| **Mean Biomass 42-48 Days Prior** | 0 | 2234 | 0,47 | 0,00 | 0,23 | 0,81 | 0,000 | 0 | A | | |
| | 1 | 75 | 0,42 | 0,02 | 0,24 | 0,78 | | 1 | | B | |
| | 2 | 10 | 0,30 | 0,02 | 0,25 | 0,47 | | 2 | | | C |

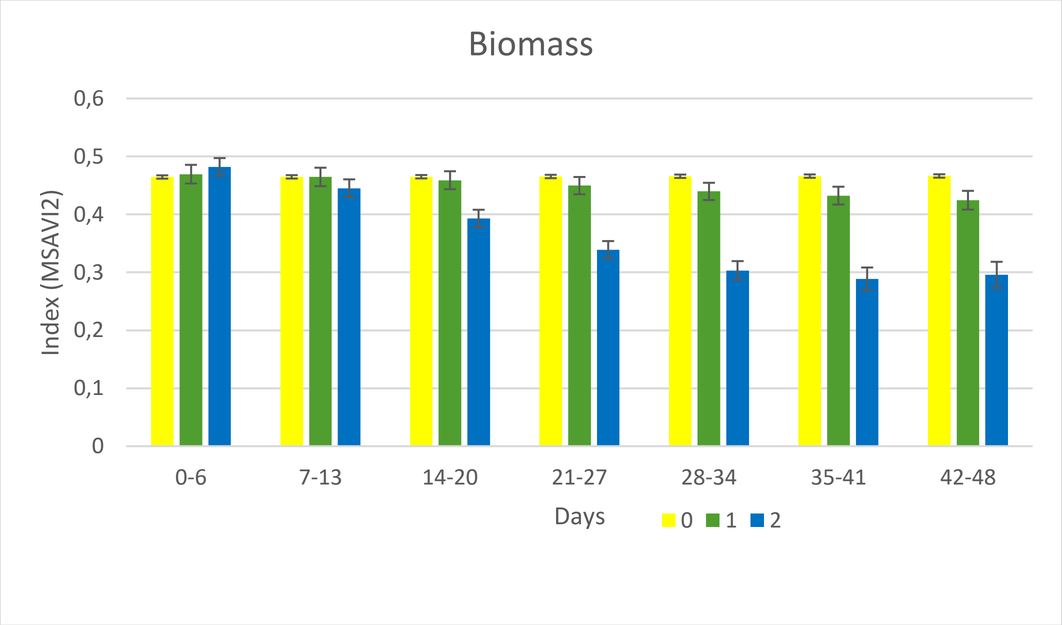


*Figure 7. On the Y-axis the daily mean biomass is displayed as an index (MSAVI2) is displayed as a percentage and the X-axis shows the period examined prior to the analysed date. Yellow bars = rank 0, days with no death due to pneumonia, green bars = rank 1 with 0.5-1.0 dead cattle due to pneumonia, blue bars = rank 2 with >1 dead cattle due to pneumonia. The black lines on the bars illustrate the standard error of the mean.*

No significant findings were made 0-6, 7-13 or 14-20 days prior to the examined shown in table 7 and figure 7. No significant findings were made 0-6, 7-13 or 14-20 days prior to the examined date. For 21-27, 28-34, 35-41 and 42-48 days low biomass increased deaths due to pneumonia ($p>0.001$).

# 6. Discussion

## 6.1 Sources of error

During the study's period 2016.01.01-2023.10.22, 168 out of 2881 days have been excluded from the study due to missing data in the mortality journals provided from OPC. Most likely this does not significantly affect the results since it is such a small proportion of the total number of days. It is possible that days without deaths are missing because there was nothing to report these days. If so, these days should have been reported as days with no deaths. However, the result of the study should not be affected very much since the proportion of days without deaths due to ECF is significantly greater (2492 days) than those with registered deaths (221 days). The study also contains enough data to get reliable results without the days with missing data.

To compensate for the long measurement interval caused by cloudy days in the biomass data, a value has been calculated by assuming the biomass values between measurements changes linearly. This most likely is not true as biomass is a living material not behaving in a linear pattern (Ali *et al.* 2016). Assuming it does is a simplification and how much this affects the results is uncertain. As the results are statistically significant the results obtained by the study are likely reliable. To minimise the bias in between the collected data, satellite pictures could have been taken more frequently to increase the number of measurements and reduce the measurement interval. However, many days during the rainy season have a high cloud coverage (Ouma & Nairobi n.d.). It is thus not certain that taking more pictures would increase the reliability of the data.

In the mortality reports supplied by OCP, differentiating between the diagnosis ECF and pneumonia was sometimes difficult for the shepherds responsible for making the diagnosis using only antemortem findings and an autopsy. Consequently, some of the animals in the mortality data may have been misdiagnosed. The shepherds can also be influenced by what they know and believe about the disease. If there is a preconceived notion that ECF increases during the rainy season, the person who decides on the diagnosis may be more inclined to give the ECF diagnosis during the rainy season. There are also other infections that may be mistaken for ECF, for example malignant catarrhal fever or other *Therelia* infections such as turning sickness, corridor disease, tzaneen disease and tropical theileriosis (Maxie 2016).

On several occasions in the mortality data, both ECF and pneumonia have been registered as the cause of death when the person who made the diagnosis was uncertain. In these cases, 0,5 deaths have been placed on both diagnoses which compromises the validity of the data somewhat since at least 0,5 of the registered death of that cattle was wrong. However, it is possible the cattle in these cases died of a combination of ECF and pneumonia.

To examine the reliability of the mortality reports used in the study, mortality data with cause of death registered as pneumonia was processed the same way as ECF. The same diagnostic analyses were performed with the environmental factors' precipitation and biomass. These were chosen because of the expectation that the result obtained should differ since the diagnoses have different courses of disease. How ECF manifests itself as a disease varies but a common disease course is an incubation period about 14 days and then death a week after onset of clinical signs (Maxie 2016). Pneumonia is a multifactorial disease with several possible agents causing the animal to fall ill (Fernández *et al.* 2020). The course of disease can therefore vary a lot. Since ECF is a tick-borne disease and has a more defined course of disease compared to pneumonia the expectation was that the analyses performed should show different results with a positive connection between heavy rainfall and high biomass when analysed with the ECF mortality data, which was the case. This could mean that when differentiation between ECF and pneumonia the diagnosis made at OPC was usually correct.

When compared to rain, ECF showed a positive connection with more deaths a couple of weeks after heavy rainfall with a peak 21-27 days prior to the date of death. P-values of <0.01 were obtained on 5 out of 7 analysed variables. This is expected due to the incubation period and course of the disease (Maxie 2016). Pneumonia, on the other hand, also showed a positive correlation with rainfall but not as strong and with a shorter time-lag, the best P-value was 0,022 and this was obtained 7-13 days prior to the date of death. As for the biomass the results obtained differ widely between the different diagnoses. While a high biomass the weeks before cattle die in ECF relates to high mortality the opposite result was obtained when analysing the pneumonia data. When analysed weekly, cattle dying due to pneumonia related to low biomass. This could be explained by the fact that pneumonia is a disease often caused by opportunists and that cattle are more likely to be affected when in poor condition (Fernández *et al.* 2020). Low biomass the week before death is likely to leave the cattle population vulnerable to infections such as pneumonia. The connection with more death when food is scarce is not found when analysing ECF indicating that factors affecting the ticks are of bigger importance than the condition of the animal. The big difference in the results obtained from the analyses of environmental factors in relation to ECF and pneumonia indicates that the diagnoses have been placed correctly.

## 6.2 Effects of environmental factors on ECF

### 6.2.1 Precipitation

The results from the performed analyses show a trend of increased rainfall prior to cattle dying due to ECF. This may be a result of the beneficial effect rain has on the tick-population (Chepkwony *et al.* 2020). In what way rain affects the vector is not investigated in this study, but it could be because they become more active, can reproduce faster or that it is easier for them to find food if the cattle prefer to be in risk areas such as bushes to escape the rain. Rainfall also stimulates grass growth; this can give the tick better conditions to succeed in finding host animals because it can climb the tall grass (Makwarela *et al.* 2023). In one study tick loads on Boran cattle were greater in the wet season compared with the dry season with the tick-loads especially high on cattle in poor condition (Chepkwony *et al.* 2021). The most distinct result from the weather analyses was obtained 21-27 days before cattle died of ECF. The disease incubation period is in general two weeks and although the time from the first symptoms to death differs widely it takes approximately one week for the disease to kill the animal (Maxie 2016). This fits well with the results and could indicate that ticks are more active and spread the disease when it rains. Although a peak was seen 21-27 days before cattle death, some variables representting time both closer to and further away also showed a strong connection with heavy rainfall and high mortality rate. This may have been because the ticks were more active during the entire rain season which stretches out over a couple of weeks (Camberlin *et al.* 2009). Considering the incubation period and course of the disease there is not enough time for ECF to cause death in cattle if the cattle was infected 7-13 days or 14-21 days prior to death. The significant results obtained 7-13 days and 14-21 days prior to death may be caused by the fact that if it rained four weeks ago and the cattle became infected this most likely occurred during the rainy season. Therefore, chances are it will continue to rain in the days or weeks to come. In one study conducted at the same study site the thesis was that mortality due to ECF would rise in association to months with heavy rainfall (Chepkwony *et al.* 2020). This was contradicted by their results, which found a negative correlation with death due to ECF and months with heavy rainfall with a lag effect of two months. Their original hypothesis is however, in good agreement with the results of this study suggesting that monthly rainfall is not a precise enough method to measure the precipitation.

Another aspect to consider is that the animals were treated with topical tick prophylaxis, the ectoparasiticide Amitaz. When it rains there is a risk that this treatment will rain off, which could leave the animals more exposed to the disease. The last couple of months of this study this treatment was oil based at OPC instead of water based; the effect of this change has not been evaluated. There is also a risk of ticks developing resistance to the ectoparasiticide (Makwarela *et al.* 2023).

### 6.2.2 Humidity

Humidity is an important factor for the ticks, when the Ixodes ticks reproduce the female lays thousands of eggs in places with high humidity to ensure their survival (Estrada-Peña & de la Fuente 2014). The results from the analyses performed show a clear connection with a higher humidity when cattle died in rank 1 & 2 compared to when no cattle died in rank 0. This relationship was significant in all investigated time spans but there was an overlap between the different rankings in 0-6 days and 42-48 days. This makes the results similar to those obtained from analysing the precipitation data but without the same clear peak at 21-27 days. This peak could have been more obscured in the analyses of the humidity data because the mean values for humidity did not vary as much as those for precipitation do. The theory was supported by the fact that the differences in the mean value between the different groups are very small. Another reason why the peak was not as clear could be because humidity is a less important factor to vectors spreading ECF compared to precipitation. There is a high correlation between humidity and precipitation; during the rainy season where the amount of precipitation increases, the humidity also increases (Omotosho & Abiodun 2007). The obtained results then pinpointed the rainy season as a risk factor for ECF but without a clear peak showing when the cattle got infected.

### 6.2.3 Dew

The analyses performed on dew and humidity were almost identical results in relation to mortality due to ECF. Dew is highly correlated with the relative humidity (Chen *et al.* 2013). Since they are so dependent on each other, analysing both might be of little value. All analysed variables gave significant differences between at least two out of three rankings 0-2. This can be because dew and humidity are of great relevance for the ticks. More likely, this result is a result of a strong correlation between increased mortality and seasonality. In the rainy season, dew and humidity increase and thus deaths in ECF should follow.

### 6.2.4 Biomass

The results from analysing the biomass data in relation to mortality in ECF showed a strong connection between deaths due to ECF and high biomass the weeks before cattle die. This relationship is in good agreement with the results from the rainfall data because the strongest connection between increased precipitation and deaths in ECF existed 21-27 days before death, during which time the biomass has time to grow (du Plessis 2001). The result shows significant results with $p<0,001$ the first weeks prior to the date of death and 35-41 days prior to death. The effect of biomass got slightly smaller 42-48 days prior to the death. Since it takes time for the biomass to grow and the rainy period only lasts a couple of weeks, these results indicate that ticks spread the disease not only during the rainy season but also when the biomass is high.

Another way of estimating biomass is to analyse the average rainfall for a time back. After continuous rainfall, the biomass increases. In the study, the average rainfall for the last 7, 30, 60 and 90 days was examined. When analysing the average rainfall for the last 7 days, no connection was found. This could be explained by the fact that it takes time for the biomass to grow, rainfall the last week is not equivalent with the biomass being high. The other investigated time spans, 30, 60 and 90 days all show the same result. Higher average rainfall was linked to high mortality due to ECF. How well this method correlates with the biomass data used in the study has not been examined.

Both analyses show the same connection between height biomass and mortality due to ECF. The reason why high biomass is so strongly linked to animal dying of ECF could be because ticks have an easier time finding hosts when biomass is high. For the tick, it is likely that grass height affects more than biomass itself, but these parameters are very strongly correlated, and it can therefore be assumed that the grass height is high when the biomass is (Dusseux *et al.* 2022).

## 6.3 Integration of cattle and wildlife

Integrating the domesticated Boran cattle with the wildlife has beneficial effects on the tourism industry which is an important income in low-income countries such as Kenya (Keesing *et al*. 2018). The integration has also proved to have some positive effects on the ecosystem. Ectoparasiticide treatment of the domesticated animals can help to reduce the overall tick population by attracting ticks and killing them when they feed. In one study tick loads were greater on wild herbivores such as zebras, elephants and rhinos than on domesticated Boran cattle (Chepkwony *et al.* 2021). This could mean that while treated domesticated cattle help reduce the abundance of ticks, the wild animals work as untreated reservoirs giving the animals unlimited amounts of food. Some animals such as the African buffalo can work as reservoirs for ECF making the disease difficult to prevent or eradicate (Olds *et al.* 2018).

## 6.4 Conclusions

The study's result shows significant connections between cattle dying of ECF and the environmental risk-factors precipitation, biomass, dew and humidity. The results of the study can be used to optimise the preventative measures in place by intensifying them when the risk of infection is high. The results also shows that the diagnosis ECF and pneumonia likely have not been confused with each other.

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# Popular science summary

ECF is a tick-borne disease caused by the protozoan parasite *Theileria parva parva* (Maxie 2016). The disease occurs mainly in central Africa where the ticks responsible for spreading the disease thrive. The main vector for the disease is the tick *Rhipicephalus appendiculatus*, a three host tick species belonging to the genera *Ixode*. It feeds on a variety of hosts including cattle and other domestic animals and is responsible for many animals falling ill (Olwoch *et al.* 2008). The number of cattle dying due to ECF is estimated to be about 1 million each year and the disease causes an economic burden of about half a billion U.S. dollars (Surve *et al.* 2023). The financial impact of ECF is caused by the effect ECF has on reduced growth, reduced production, cost of prophylactic treatment and treatment for acute disease. To reduce the occurrence of ECF prophylactic treatment directed towards the ticks is used (Olwoch *et al.* 2008). The tick population and geographic distribution is affected by environmental factors such as humidity, temperature and precipitation and it is important to understand how these factors affect the spread of ECF to develop and use prophylactic treatment in an effective way (Makwarela *et al.* 2023). However, it can be difficult to exclude risk factors since many are highly associated with key resources for the hosts making the ticks unavoidable (De Garine-Wichatitsky 2000). The most common prophylactic treatment used is topical use of antiparasitic pharmaceuticals to reduce the number of ticks on the animals (Olwoch *et al.* 2008). This treatment is an economic burden for the farmers and there is a problem with ticks developing resistance. Other potential difficulties with the treatment in use are movement of animals, lack of adequate treatment stations and the environmental burden caused by using pharmaceuticals.

In this study precipitation, biomass, dew, temperature and humidity were analysed together with mortality data for ECF. The environmental factors have been chosen not because of the way they interact with the disease but how they might affect the ticks crucial for spreading ECF. It takes about two weeks before the cattle starts to show symptoms after being infected and then some time for the disease to kill the cattle if the animal does not recover instead (Maxie 2016). Therefore, the study is designed to assess the connection between the environmental factors the weeks before the cattle died. This is because it is the time before infection of the animals that are of interest.

The study has been conducted at the Ol Pejeta Conservancy in Laikipia, Kenya. In this part of Africa ECF is a big problem for the cattle industry (Surve *et al.* 2023). The mortality and precipitation data used was obtained from OPC. Data for humidity, temperature and dew was obtained from the digital weather database Visual Crossing and the biomass data was obtained from SLU using satellite imaging. The data was processed day by day and the mean values from one week was used in the analyses with a time lag to research the parameters a time back. This was done to investigate the period before infection. Results obtained by analysing the data show a positive connection between precipitation, humidity, dew and biomass. Higher values of these environmental factors relate to cattle dying due to ECF. No statistically significant results were obtained when analysing death due to ECF in relation to temperature.

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