



# **Activity patterns of African buffalo (*Syncerus caffer*) and the transmission of East Coast Fever to domestic cattle in Laikipia County, Kenya**

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*Aktivitetsmönster hos afrikansk buffel och smittöverföring av East Coast Fever i Laikipia-regionen, Kenya.*

Alva Sellgren Molander

Degree project/Independent project • 30 credits

Swedish University of Agricultural Sciences, SLU

Faculty of Veterinary Medicine and Animal Science

Veterinary Medicine Programme





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Alva Sellgren Molander

**Supervisor:** Jens Jung, Swedish University of Agricultural Sciences, Department of Animal Environment and Health  
**Assistant supervisor:** Maria Andersson, Swedish University of Agricultural Sciences, Department of Animal Environment and Health  
**Examiner:** Jenny Yngvesson, Swedish University of Agricultural Sciences, Department of Animal Environment and Health

**Credits:** 30 credits  
**Level:** A2E  
**Course title:** Independent Project in Veterinary Medicine  
**Course code:** EX0869  
**Programme/education:** Veterinary Medicine Programme  
**Course coordinating dept:** Department of Clinical Sciences

**Place of publication:** Uppsala  
**Year of publication:** 2022

**Keywords:** Buffalo, *Syncerus caffer*, East coast fever, ECF, *Theileria parva*, Corridor disease, CD, Kenya, Laikipia

**Swedish University of Agricultural Sciences**  
Faculty of Veterinary Medicine and Animal Science  
Department of Animal Environment and Health



## Abstract

In this study we examined the potential connection between East Coast Fever (ECF) and the local activity pattern of African buffalo in the Ol Pejeta Conservancy (OPC), in Laikipia county, Kenya. Buffalo activity was examined at OPC's wildlife corridors, which work as the only gates for the animals to pass in and out of the area, by looking at approximately 32 000 pictures taken by infra-red cameras during the year 2019. In these pictures, 2859 buffaloes were counted. A correlation between number of buffaloes and the number of pictures taken was then tested and proven highly positive. We then proceeded to test if there was a liaison between the number of buffalo-pictures for the years 2016-2019 and the ECF-related deaths during the same years.

The results showed that there was no correlation between increased buffalo activity and increased cases of ECF. However, a correlation between number of buffaloes and numbers of ECF cases could still exist, but the methods used in this study are suspected to not be sensitive enough to find one. More detailed research is needed about the buffaloes' movements inside the reserve, in relation to cattle movement. In this study, we had access to the number of ECF-related deaths. For a correct picture, the total amount of ECF-cases is needed to be able to examine a liaison. The buffaloes' movements should also be examined in relation to rainfall since rainfall affects buffaloes' movement, and it can also influence the number of ECF-cases.

We also tested the interval for spraying the cattle with anti-tick treatment. The interval used at the OPC was considered necessary under the current circumstances for the trial. During the testing period there had been more rainfall than usual. Different intervals should be tested during different weather conditions to see if the interval can be prolonged during drier seasons.

*Keywords:* Buffalo, *Syncerus caffer*, East coast fever, ECF, *Theileria parva*, Corridor disease, CD, Kenya, Laikipia

## Sammanfattning

I denna studie undersöktes det om det finns ett samband mellan buffelaktivitet och antalet dödsfall hos kor i sjukdomen East Coast Fever (ECF) på Ol Pejeta Conservancy (OPC) i Laikipia County Kenya. Buffelaktiviteten registrerades genom att studera runt 32 000 bilder på bufflar, tagna av infra-röda kameror som är uppsatta längs OPC:s så kallade korridorer, vilka fungerar som de enda portar för djuren att passera in eller ut i området. På bilderna kunde 2859 bufflar räknas. Vi testade om det fanns en korrelation mellan antalet bilder och bufflar, vilket tydligt fanns. Efter detta fortsatte vi med testa om det fanns ett samband mellan antalet bilder på bufflar (buffelaktivitet) och antal dödsfall i ECF.

Resultaten visade att det inte fanns något samband mellan ökad buffelaktivitet och ökat antal ECF fall på OPC. Ett samband kan dock ändå finnas, men de metoder som användes i denna studie var inte tillräckligt känsliga för att hitta detta. Det behövs mer detaljerade studier där bufflarnas rörelser inom området, och rörelser i relation till kornas undersöks. I studien hade vi endast tillgång till antalet dödsfall för ECF, och inte totalantalet ECF-fall, vilka bör inkluderas för en mer korrekt bild. Bufflarnas rörelser bör också undersökas i relation till nederbörd, då detta påverkar både bufflar och fall av ECF.

I studien testades även intervallet för när OPC sprayar sina kor med antiparasitära medel. Spray-intervallet som användes under tiden för studien ansågs nödvändig under de omständigheter som rådde. Under perioden var det mer nederbörd än vanligt. Intervallet bör testas under flera olika väderomständigheter för att se om intervallet kan förlängas under torrare perioder.

*Nyckelord:* Buffel, *Syncerus caffer*, East coast fever, ECF, *Theileria parva*, Corridor disease, CD, Kenya, Laikipia

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## Abbreviations

CD	Corridor Disease
DMR	Daily Mortality Reports
ECF	East Coast Fever
ITM	Infection and Treatment Method
OPC	OI Pejeta Conservancy
SLU	Swedish University of Agricultural Sciences



# 1. Introduction

East Coast Fever (ECF), also known as Theileriosis, is a disease that can be caused by various species of the protozoa *Theileria* (Taylor *et al.* 2007). *Theileria parva* causes ECF in predominantly eastern and central Africa (Taylor *et al.* 2007, Nene *et al.* 2016). The disease is present in 11 countries in Africa, and an estimate of 1 million cattle die every year which comes with economic losses exceeding USD 300 million annually (Gachohi *et al.* 2012, ILRI 2014).

East coast fever is a disease of importance to farmers in endemic areas since it leads to great losses in production and economy, as well as decreased animal welfare. Ol Pejeta Conservancy works with conservation of wildlife in a wildlife-livestock integration system. The cattle at the ranch share their pastures with the African buffalo (*Syncerus caffer*) which serves as a reservoir for *T. parva* (Taylor *et al.* 2007). It is important to investigate how this way of keeping animals affects prevalence of disease, together with differences in genetic host factors. Ol Pejeta runs the biggest beef farm in Kenya with 6 000 heads of cattle that share the area with about 3000 wild buffaloes (van Aardt 2021).

ECF in Boran cattle is a problem at Ol Pejeta despite the prophylactic measures such as spraying the animals approximately every 5<sup>th</sup> – 7<sup>th</sup> day with anti-tick treatment, and vaccination trials (van Aardt 2021). At OPC cattle are exposed to wild buffaloes and these two species share pasture grounds. According to earlier research the wild African buffalo facilitates mutation and mixing of different strains of *T. parva*, which could potentially be the cause for severe disease, even in vaccinated cattle.

## 2. Background

### 2.1. *Theileria* spp. and East Coast Fever (ECF)

Globally there are many different kinds of *Theileria* species (Taylor *et al.* 2007). *T. parva* and its two subspecies *T. parva parva* and *T. parva lawrencei* are the main causes for East Coast Fever in cattle in Africa (Taylor *et al.* 2007). It is a tick borne parasite and transfers to cattle most commonly with the brown ear tick *Rhipicephalus appendiculatus* (Taylor *et al.* 2007, Nene *et al.* 2016). The brown ear tick serves as an intermediate host that ingest erythrocytic merozoites; these develop into primary and secondary sporoblasts in the adult tick (Taylor *et al.* 2007). They start to produce sporozoites that are released into the tick's saliva and can be transferred to cattle when the tick sucks blood (Taylor *et al.* 2007). The sporozoites quickly inoculate and enter lymphocytes in an associated lymph gland, usually the parotid gland (Taylor *et al.* 2007). The infected lymphocyte transforms to a lymphoblast and divides with a ten-fold rate, at the same time as macroschizonts develop synchronously with the cells (Taylor *et al.* 2007). These produce microschantons that enter erythrocytes (Taylor *et al.* 2007). This eventually leads to a massive lymphocytolysis and depressed leucopoiesis (Taylor *et al.* 2007).

The incubation period for Theileriosis varies between 8-12 days (OIE 2020). The animals often show enlarged lymph nodes, fever (40–41.7°C), increased respiratory rate, dyspnea and/or diarrhoea, often blood stained (Taylor *et al.* 2007, OIE 2018). The animals general condition is impaired, they become anorexic and milk production decreases (Taylor *et al.* 2007). Recumbence and death usually occurs within 3 weeks (Taylor *et al.* 2007). Animals that recover become carriers of the parasite and may spread it to other animals (OIE 2020).

The mortality rate varies with several factors, such as parasite strain, infective dose and host features (Nene *et al.* 2016, OIE 2018). Some breeds of indigenous African cattle suffer milder forms of ECF with a lower mortality rate than breeds that more recently have been introduced to the continent (Morrison *et al.* 2020). The mortality rate can go up to 100% in the most susceptible breeds, but in indigenous cattle it can be as low as 3% (OIE 2018, 2020). *Bos taurus* of European breeds cattle usually get a very severe form of ECF, but mortality is often high among young zebu cattle in pastoralist systems as well (Giulio *et al.* 2009).

The wild African buffalo carries the parasite without showing signs of clinical disease and is an important way for transmission to domestic cattle (Taylor *et al.* 2007; Nene *et al.* 2016; Morrison *et al.* 2020). Taylor *et al.* (2007) explain that the subspecies *T. parva lawrencei* is transmitted from the African buffalo and becomes indistinguishable in its behaviour from the other subspecies *T. parva parva* following several passages in cattle (Taylor *et al.* 2007). It has been discussed if these two subspecies are the same species because of their molecular similarities and more recently the parasites are usually referred to as *T. parva* of cattle or buffalo origin (Morrison *et al.* 2020; Cook *et al.* 2021).

Buffalo-derived parasite strains are more diverse than cattle-derived parasites and *T. parva* derived from wild buffalo usually causes a more severe and acute form of ECF, referred to as corridor disease (CD) (Nene *et al.* 2016; Magulu *et al.* 2019; Morrison *et al.* 2020). The buffalo strains also face difficulties in circulating in cattle, which Morrison *et al.* also state in their review from 2020.

In CD there are also lower levels of schizont-infected leukocytes in lymphoid tissues, and very few to no piroplasms in erythrocytes (Maritim *et al.* 1992; Morrison *et al.* 2020), compared to ECF where parasitosis of schizonts, and number of piroplasms is high (Maritim *et al.* 1992; Taylor *et al.* 2007). The low levels of parasitemia decreases the chances for the parasite to transfer to other ticks. There are no clinical useful diagnostic methods though to distinguish between CD and ECF. (Cook *et al.* 2021).

## 2.2. Prevention and treatment options

There are three main methods for controlling ECF, these are; tick control, live vaccination through an Infection and Treatment and Method (ITM) vaccine, and treatment of sick animals (Katingi 2011).

*Tick control* includes measures as dipping pools (the cow goes into a pool of water with anti-tick treatment), spraying the cow with a similar treatment, and manual tick removal (Kimaro *et al.* 2017). Acaricides are a group of substances commonly used to kill ticks, however it is not a sustainable method since it is expensive, environmentally hazardous, and the ticks will in time develop resistance (OIE 2020). At Ol Pejeta Conservancy they spray their animals approximately every 5<sup>th</sup> to 7<sup>th</sup> day with acaricides (van Aardt 2021).

*Vaccine* – When vaccinating against ECF a live vaccine is used containing a mixture of parasite strains and then treating the animal (Katingi 2011; Nene *et al.* 2016; OIE 2018). This is a so-called infection and treatment method (ITM) (Magulu *et al.* 2019; Allan *et al.* 2021). The most commonly used vaccine, the Muguga cocktail, contains a mixture of stabilates from three different strains of *T. parva*; these are *T. parva* Muguga stabilate, *T. parva* Kiambu-5 stabilate and *T. parva*

Serengeti transformed stabilate (Katingi 2011; Nene *et al.* 2016). The animals become inoculated with an appropriate dose of viable sporozoites subcutaneously, at the same occasion they are treated with a long acting formula of tetracycline that reduces the severity of the infection to a mild form that is supposed to be controlled by the host's immune system (OIE 2018). Some *T. parva* causes very mild infections and can be used for inoculation without tetracycline treatment (OIE 2018).

The Muguga cocktail has been used since the 1970's but faces commercial production problems (Nene *et al.* 2016). Since the animals are being exposed to viable parasites there is a bigger risk of an uncontrolled infection, therefore it is important to thoroughly control the quality of the stabilate and dosage. This ITM vaccine is being used successfully in the field despite that there are stabilates that can give rise to severe disease (OIE 2018). Except that it might cause death, the Muguga cocktail is expensive, difficult to produce, requires a liquid cold chain for storage (Nene *et al.* 2016) and the animals can become carriers of the vaccine strains (Giulio *et al.* 2009; Lacasta *et al.* 2018). The need for antibiotic use at the time of administration is another reason for why this vaccine needs to be improved. The spread of antibiotic resistance is a well-known problem, and the negative effects are already clearly showing in medical care for both humans and animals.

Since the *T. parva* strains vary in different areas the effectiveness of vaccine might vary with which stabilate is used (Giulio *et al.* 2009). Because of this there are other *T. parva* stocks being used in different areas for immunization besides the stabilates in the Muguga cocktail (Giulio *et al.* 2009, Nene *et al.* 2016). In Morrison *et al.* (2020) the authors reviewed evidence for the cocktail to not give cross protection between *T. parva* of cattle and buffalo origin, for buffalo derived parasites it seems to fail to give complete protection. A study at Ol Pejeta Conservancy showed no difference in mortality in vaccinated cattle that had seroconverted, and a control group that were exposed to buffalo-derived *T. parva* (Sitt *et al.* 2015). Only six out of 24 animals survived, of which three were vaccinated and three were from the control group (Sitt *et al.* 2015).

There is a demand on finding new more sustainable vaccines and treatment methods (OIE 2018). There has been extensive research on developing non-live vaccines, where many focuses on the gene coding for the sporozoite specific protein p67 (Nene *et al.* 1996; Nene & Morrison 2016; Lacasta *et al.* 2018). Experimentally p67 vaccines seem to give approximately 50% immunity to the homologous and heterologous sporozoite challenge (Nene & Morrison 2016). Enough protection is however not seen by one vaccination, and at least three doses are needed which is not practical in field conditions (Nene & Morrison 2016; Lacasta *et al.* 2018).

Other commercial drugs that are used for treatment are chemotherapeutic drugs such as buparvaquone and paravaquone (Kimaro *et al.* 2017, OIE 2020). Imidocarb and tetracycline on their own are also used as treatment but seems to have poor response in more serious cases (OIE 2020)



In a study in Monduli District, Tanzania, a cattle owner survey was carried out about recognition and treatment of ECF among the owners. Besides the treatment options mentioned earlier they also reported using non-effective antibiotics like tylosin and penicillin (Kimaro *et al.* 2017).

It is possible to eradicate the disease, but this is very difficult when buffalos keep transmitting the infected ticks. A review of Nene *et al.* (2016) mentions that South Africa managed to eradicate the disease through a strict control of cattle movement, surveillance with slaughter of infected cattle, and fencing to restrict buffalo to game parks, but that occasional outbreaks of buffalo-derived *T. parva* disease still occur. Hence, in areas with both buffaloes and cattle ECF remains a problem.

### 2.3. The livestock of Ol Pejeta Conservancy and their wildlife/livestock integrated system

The Ol Pejeta Conservancy (OPC) is located in central Kenya's Laikipia County. They work to conserve wildlife and manages research and community development (Ol Pejeta Conservancy 2021b).

There are around 6000 purebred Boran cattle in the OPC (Ol Pejeta Conservancy 2021a). They also have around 200 Ankole cattle in the conservancy, an old East African breed of African *Bos taurus* and of *B. indicus* origin that is much more resistant to ECF than Boran, and a smaller number of Angus cows (van Aardt, 2021). Another 1500 domestic cattle owned by other farmers or organizations are occasionally also grazing on OPC's grounds. The herds do not mix.

The pastures of the domestic cattle are shared with wildlife including predators and buffaloes. To protect cattle from predators, they are guarded in enclosures made of high metal fences at night. The benefits of an integrated wildlife and livestock system are that it helps breaks up soil, fertilizes the ground and camera traps in the livestock-grazed areas have recorded significantly more herbivores compared to other areas (Ol Pejeta Conservancy 2021c).

The challenges the OPC says to face with this system is predation from big cats and tick borne diseases, particularly from buffaloes (Ol Pejeta Conservancy 2021c). The buffaloes migrate and can walk freely in and out of the reserve. This means that strains of *T. parva* can mix and new genetic parasite material can be introduced to resident parasite strains with the buffaloes' migration. There is a risk that the mixing of parasite material from different sites will give rise to resistance against the different treatment methods (Giulio *et al.* 2009).

An average herd size is since 2019 around 250 cows and 250 calves. If it is a herd of heifers and steers the number varies between 250-300 animals if the herd of heifers and steers. Limitation for herd size is water. Before 2019 the average herd

size was 100 adult animals. Since increasing the herd size they have not noticed any difference in ECF cases, they are aware of that picking out ECF cases might be harder though.

Age groups as categorised by the OPC:

- Calves: 0-8 months.
- Heifers: 8 months to 2 years.
- Steers: 8 months to years
- Cow: after calving, up to 10 years.
- Bull: In general 2.5 years and above. From what age depends on the individual. Usually, they are ready to breed from 2.5 years.

There are two main calving seasons, one in November to December, and one in April to May, but there can be births all year. The calves are weaned around eight months. The steers are castrated at an age of 7 months.

OPC slaughter their animals in their own slaughterhouse. Slaughter takes place two times/week, 20 animals/time. Males are slaughtered around 3 years of age, cows around 10 years, depending on the animal.

## 2.4. Buffalo ecology

The African buffalo is found in many African countries. The population trend is decreasing and they are losing much of their habitats to mainly residential and commercial development and agriculture and aquaculture (IUCN, Red List of Threatened Species 2020). In Kenya savannah buffalo (*Syncerus caffer caffer*) is the dominating subspecies of African Buffalo (Cornelis *et al.* 2014). It inhabits many different types of habitats, from rain forests to dry savannas (Cornelis *et al.* 2014; IUCN, Red List of Threatened Species 2020).

Savannah buffalo can weigh between 500 to 800 kg (Megaze *et al.* 2013). They usually move in herds and herd size varies between 10-1654 individuals with the mean herd size for Central African buffalo being 45 individuals (Cornelis *et al.* 2014). Larger herds contain around 150 animals or more (Cornelis *et al.* 2014). Factors deciding herd size are age, sex, access to food, soil components and season (Winnie Jr. *et al.* 2008; Megaze *et al.* 2013; Cornelis *et al.* 2014).

For the savannah buffalo the availability of surface water and cover are listed as determining factors for habitat use, usually they move where surface water is available within 20-40 km year round (Cornelis *et al.* 2014). Daily movement differs between 1-15 km (Cornelis *et al.* 2014).

Buffaloes prefer habitats with access to mud baths and sweet grass species (Furstenburg 2010). Suitable forage material can for example be found in woodlands such as miombo (*Brachystegia* spp.), mopane (*Colophospermum*

*mopane*), acacia (*Acacia* spp.) and *Baikiaea* spp. (Cornelis *et al.* 2014). OPC covers 360 km<sup>2</sup> and many different land types can be found within its borders. The three major habitats are open grasslands; tree savannah with mainly *Acacia drepanolobium*, and *A. xanthophloea*, and tree savannah with mainly *Euclea divinorum* (Mutunga Kavwele 2017).

Migration and home range habitat vary greatly between herds in different areas depending on local conditions (Naidoo *et al.* 2012; Megaze *et al.* 2013; Dublin *et al.* 2015; Roug *et al.* 2020). Herds can be migratory, partial migratory where only a fraction of the population migrate and non-migratory (residents) (Naidoo *et al.* 2012; Roug *et al.* 2020). Naidoo *et al.* 2012 observed in their study that individuals never switched strategies between seasons. They also noticed a type of movement pattern for groups that they called expanders, who expand their home territory during drought.

The number of buffaloes at the OPC have steadily increased during the years (van Aardt, 2021). An estimate of 3000 buffaloes now graze on the conservancy. According to Richard van Aardt they have not registered a pattern in the buffaloes' migration movements in and out of the conservancy. The number of buffaloes seems to be static, or possibly increasing. They have noticed that the buffaloes like to graze where the cattle have been grazing.

### 3. Aims of study

It is important to map the risk factors of ECF and why some cows become more affected than others, to be able to understand which measures are necessary to prevent disease.

The purpose of this study was to investigate the possible connection between the local migration of wild African buffalo, and the prevalence of ECF in domestic cattle in OPC.

This was investigated by first mapping activity of buffaloes in the conservancy by looking at pictures taken by cameras put up at so called corridors which are used by animals as entries into the conservancy. After concluding that there is a relation between number of pictures taken, and the number of buffaloes, the total number of pictures was compared to the numbers of deaths of cattle caused by ECF throughout the years 2016-2019.

Our hypothesis was that more buffalo activity would be associated with a higher number of ECF deaths among the cattle.

We also tested and compared two methods of recording presence of buffaloes. One method was recording number of pictures with buffaloes present, the other method was counting the number of buffaloes on each picture.

In this study we also tested the interval for spraying the cattle with acaricides by counting ticks on the animals before and after spray to decide whether the spray interval was necessary or could be prolonged.

## 4. Methods

This study was done by a collaboration with staff from SLU and OPC.

### 4.1. Study site

The Ol Pejeta Conservancy is situated in Laikipia County, Kenya. The conservancy is around 360 km<sup>2</sup> and is surrounded by a 120 km long, solar powered electrical fence, with the exception of a number of openings, so called corridors (Ol Pejeta Conservancy 2022). The corridors work as gates and are designed to let all animal species walk in and out of the reserve, with the exception of rhinos which they want to keep within the fence for protection. The corridors therefore have features such as poles and rocks that are difficult to cross for rhinos but not for any other species. (Ol Pejeta Conservancy 2022). The corridors have infra-red cameras installed that takes pictures of moving object that crosses the gates. There are three corridors in total named 1, 2 and 3. Currently two corridors are used, number 1 and 2.

The conservancy is divided into many small sections. To avoid conflicts with farmers, the corridors are located at the northern border of the park since the area north of OPC has less farmland than in the other directions.

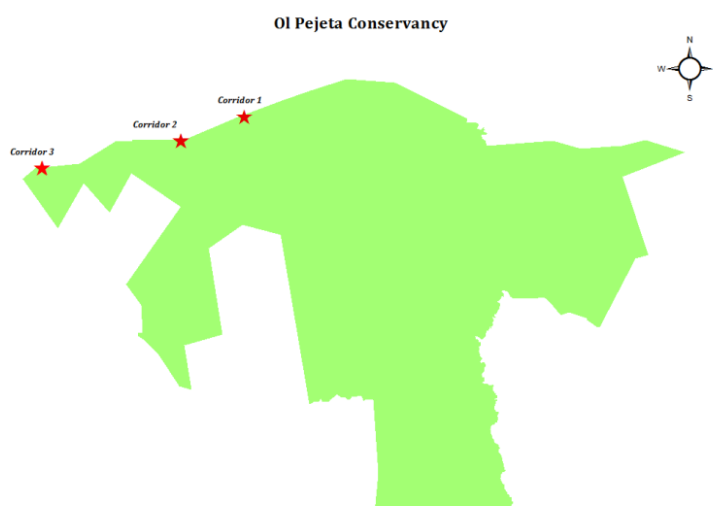


Figure 1. Map of OPC with the corridors pointed out. Picture by Grabbe, Emelie 2018

Corridor 1 is 183 meters long, it has six cameras labelled A-F. Corridor 2 is 34 meters long with three cameras labelled A-C. Corridor 3 is also 34 meters long with 3 cameras. Corridor 3 was closed during the timespan for the study and is not included in this work.

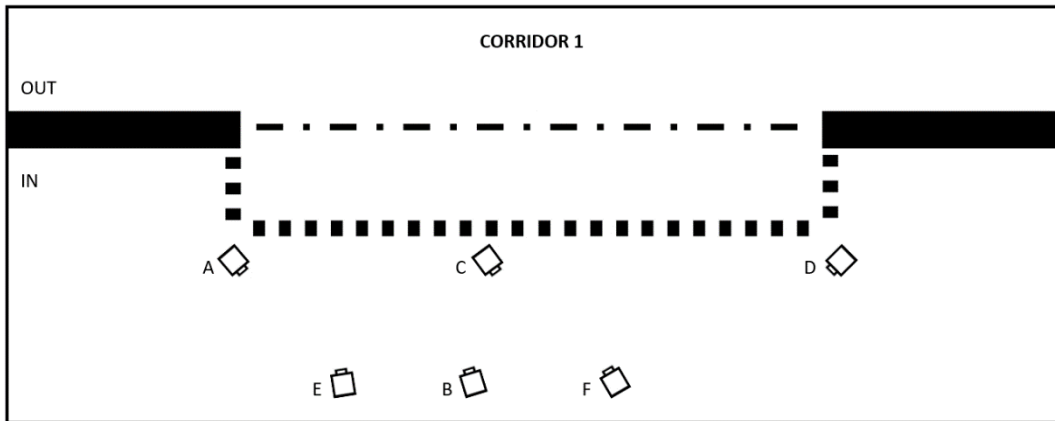


Figure 2. Corridor 1, camera placement.

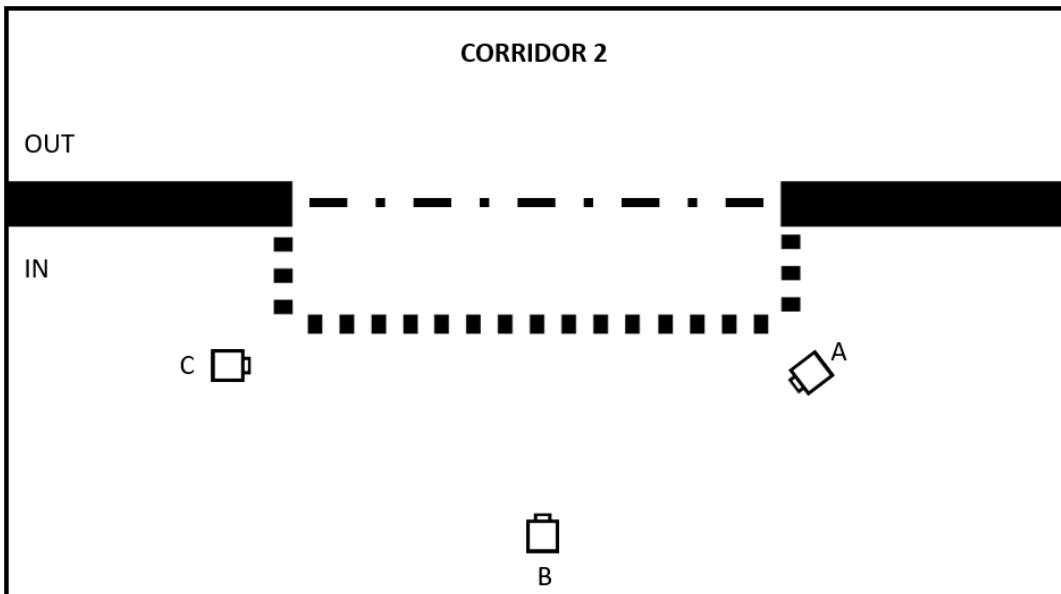


Figure 3. Corridor 2, camera placement.

## 4.2. Daily mortality reports

The cattle managers of OPC diagnosed and recorded cattle that died from East coast fever since 2015 with date, type of cattle (calf, steer, heifer, cow) and location (part of OPC). We then analysed these data regarding several factors, in my case buffalo migration.

The OPC keep records of how many cows that are lost every year due to different causes, these are called “*Daily mortality reports*” (DMR). Every death is registered in the file. There are many causes included, for example predation, trampling, and different diseases. Since this study is about East coast fever, only causes of mortality related to infectious diseases will be mentioned. The causes related to specific infectious diseases mentioned in the DMRs are; East coast fever (ECF), anaplasmosis, foot and mouth disease (FMD), pneumonia.

Since there are limited methods to differentiate between ECF and CD, and the two diseases are caused by the same parasite, ECF is here chosen as the general term describing *T.parva* infection from both buffalo-derived strains and cattle derived strains.

Diagnosis of ECF is done by a veterinary technician employed by the OPC. The main clinical signs that are used for diagnosis are raised temperature, swollen submandibular lymph nodes and panting. If the animal succumbs to disease or is found dead a necropsy is performed. Post mortem findings that leads to ECF diagnosis is:

- Frothy trachea
- Froth inside lungs
- Enlarges submandibular lymph nodes and enlarged prescapular lymph nodes
- Petechia under the tongue, heart and lungs.

At least three are needed to conclude a ECF diagnosis.

The DMR’s are made by the OPC staff in Excel, one excel file per year. We had access to files from January 2016 to September 2021. Every yearly file is divided into 12 sheets, one sheet per month. At the bottom of the file there is a monthly mortality summary. Every sheet has 10 columns (A-J) arranged in the following order; date, category, brand no., chip no., no. head, cause, herder, location, reported by, remarks.

	A	B	C	D	E	F	G	H	I	J
1	DATE	CATEGORY	BRAND NO.	CHIP NO.	No. HEAD	CAUSE	HERDER	LOCATION	REPORTED BY	REMARKS
2	2017-01-12	Calf			1	Predation	Ngasike	Airstrip	Rose	
3	2017-02-12	Steer	Tag 5415	613928	1	Scouring	Jackson	Murera Ndonga	Chelule	
4	2017-02-12	Calf	9676	600431	1	Trampled	Barusey	O-Whisky	Rose	
5	2017-03-12				0					
6	2017-04-12				0					
7	2017-05-12	Cow(Fresian)	37		1	ECF	Ewaton	Airstrip	Rose	Pneumonia
8	2017-06-12	Calf	9216	602767	1	Weak	Ewaton	Airstrip	Rose	Not suckling
9	2017-06-12	Calf			2	Suspected predation	Kilwa	Nyumba nne	J.Mathenge	
10	2017-07-12				0					
11	2017-08-12	Calf	323	595619	1	Trampled	Ngasike	Nyumba nne	C.Mathenge	
12	2017-09-12				0					
13	2017-10-12	Cow	8163/1507		1	Stomatitis	Kalula	Airstrip	C.Mathenge	
14	2017-11-12	Calf	4463		1	Still birth	Ewaton	Airstrip	C.Mathenge	
15	2017-11-12	Calf	4383		1	Trampled by Zebras	Ewaton	Airstrip	C.Mathenge	
16	2017-12-12				0					
17	13/12/2017	Calf	5009		1	Still birth	Ewaton	Airstrip	Ewaton	
18	14/12/2017				0					
19	15/12/2017	Calf	4553		1	Still birth	Ewaton	Airstrip	C.Mathenge	Mummified foetus
20	16/12/2017				0					
21	17/12/2017				0					
22	18/12/2017	Calf	L20		1	Tramped/Weak	Ewaton	Airstrip	C.Mathenge	
23	19/12/2017	Calf	9270		1	Still birth	Ewaton	Airstrip	C.Mathenge	
24	19/12/2017	Calf	1022		1	Abortion	Lungoo	Mlima Chui	Tika	
25	20/12/2017	Calf		601262	1	Bloat	Ajihot	Nyumba nne	C.Mathenge	
26	20/12/2017	NRT Steer			1	Debility	Dictor	Kambi faru	Mukundi	
27	21/12/2017	Calf			1	Trampled	Ajihot	Nyumba nne	C.Mathenge	
28	22/12/2017	Calf			1	Scouring	Ngasike	O-Whisky	C.Mathenge	
29	23/12/2017	Calf			1	Trampled	Kindelel	Nyumba Tano	Patrick	
30	24/12/2017	Steer	068/6		1	Naval illness	Lopeiyo	Kamok	C.Mathenge	
31	24/12/2017	Calf			1	Predation( lion Night)	Larpei	Nyumba Tano	Patrick	
32	25/12/2017	Calf	9411	587559	1	Predation(Leopard)	Abdi	Kamok	C.Mathenge	
33	26/12/2017				0					
34	27/12/2017				0					
35	28/12/2017				0					
36	29/12/2017	Calf	1395	520193	1	Ruminal infection	Ajihot	Nyumba nne	Patrick	
37	30/12/2017				0					

Figure 4. Screenshot of DMR 2017.

To be able to analyze the ECF incidence and any potential connection to other factors one single excel file was created from the Excel files 2016 to 2021 with only one sheet with every date from 01-01-2016 to 30-09-2021. Every ECF case recorded from the previous files was inserted on the correct date. We used the following columns:

DATE, ECF, ECF/OTHER, DEATH TOT, HEIFER, COW, CALF, BULL, STEER. LOCATION, REMARKS.

### 4.3. Buffalo movement in and out of the conservancy

To examine buffalo activity in the reserve, pictures from the cameras were studied. Pictures were collected from the automatic cameras and sorted by a field assistant at the OPC. The pictures were sorted by species, corridor and date.

In total there were about 164 000 pictures from the years 2016 and 2019 sorted into the buffalo category. There was not enough time to count buffaloes for all these pictures, therefore a fraction was chosen to test if there was a correlation between number of pictures and numbers of buffaloes. After we saw that there was a correlation we chose to compare number of pictures in relation to ECF deaths over a longer period.



### 4.3.1. Buffalo-count in Camelot

Buffalo-count was step one in order to decide if there was a correlation between number of pictures and numbers of buffaloes. For sorting and counting buffaloes only pictures from the year 2019 were chosen. In total 32 480 pictures were sorted in the application Camelot (Camelot 2022).

Pictures were transferred to the application into folders sorted by corridor and camera. Folders were named corridor 1, 2 and 3. Cameras were named after number of corridor and the letters A-E. For example, camera A in corridor 1 was named 1A.



Figure 5. Screenshot from Camelot, library.

The pictures within every folder were examined. Usually there were more than one picture for every single buffalo, or group of buffaloes, therefore the pictures were sorted in bunches. For every bunch of pictures certain parameters were noted. In this study every note for one or more pictures is called a sighting.

For every sighting the following parameters were noted.

- Species
- Calves spotted in group
  - o Yes
  - o No
  - o Unknown
- Comment
- Direction
  - o In
  - o Out
  - o Along
  - o Unknown
- Group size

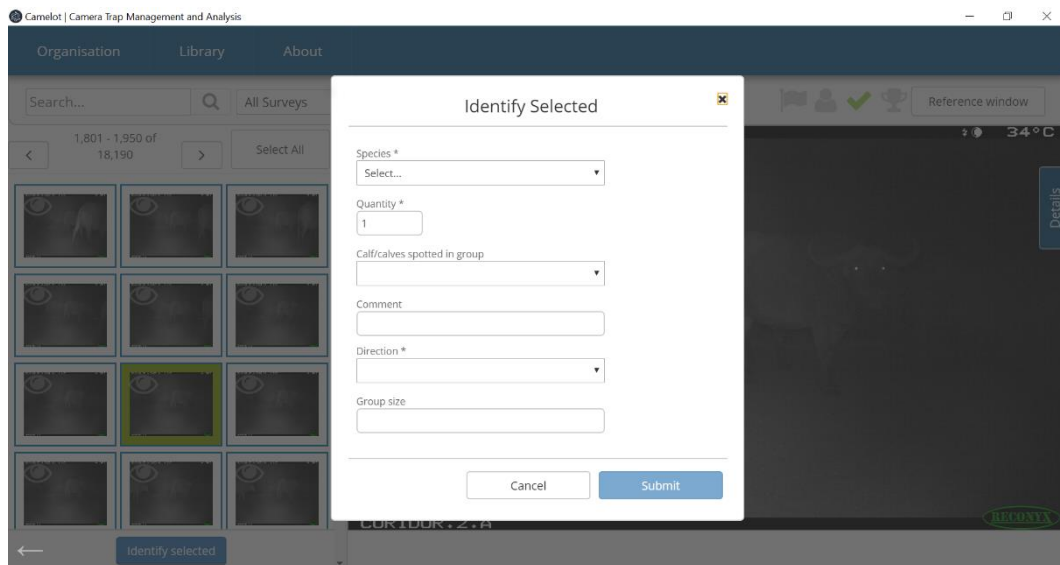


Figure 6. Screenshot from Camelot, identification of sighting.

In total 32 480 pictures were uploaded to the Camelot application, the picture upload was divided into two uploads. The number of buffaloes were counted as groups, and a sighting was made for each group. The sightings were converted to an Excel file and the data was double checked.

Buffaloes usually migrate in herds and the pictures often contained many buffaloes and many images were taken of the same animals. The buffaloes were therefore counted in series of pictures.

It was not possible to count every individual in the pictures and the numbers stated in this study is not the exact number of buffaloes walking in.

In situations when animals are grazing and walking back and forth in front of the camera, there is a risk that the animal might have been double-counted. To avoid double-counting for large groups, a direction from which way to count was decided, depending on from which side the larger part of buffaloes were coming into the picture. If the whole group seemed to be moving to the right and some animals came in from the right these were not counted.

There is also a risk of missing animals since there might be more animals in the group than the cameras caught. A majority of pictures were taken during the night so those buffaloes standing out of reach of the infra-red flash could not be seen. Sometimes animals were also standing too close to the camera, covering the image and thus covering what is happening in the background. If there were animals behind this first buffalo, these animals were simply not counted.

Pictures taken by camera D were ignored during the second upload because of poor quality. Therefore, pictures from camera D were not included in step two when comparing pictures from folders and camera (see methods 4.3.2. below).

The correlation between number of buffalo pictures and number of individual buffaloes was analysed with the Pearson correlation test.

#### 4.3.2. Pictures by folder and camera

Compiling information about number of pictures by folder and camera was step two. The pictures from OPC were downloaded and sorted into folders by a technician of OPC. The buffalo pictures were sorted into folders by the date for download and by camera. The downloads were usually done once a week however this is was not always followed. The interval for downloads varied between three and 28 days, with seven being the most common interval.

Data was collected by going through every camera folder in the buffalo category noting how many pictures there were between the download-dates. A mean value for pictures taken per day was then calculated for every period.

	A	B	C	D	E
1	Date	Total ECF deaths	Mean pics/dates	Days between upload	
2	2016-01-01	0	9	8	
3	2016-01-02	0	9	8	
4	2016-01-03	0	9	8	
5	2016-01-04	0	9	8	
6	2016-01-05	0	9	8	
7	2016-01-06	0	9	8	
8	2016-01-07	0	9	8	
9	2016-01-08	0	9	8	
10	2016-01-09	0	13	7	
11	2016-01-10	1	13	7	
12	2016-01-11	0	13	7	
13	2016-01-12	0	13	7	
14	2016-01-13	0	13	7	
15	2016-01-14	0	13	7	
16	2016-01-15	0	13	7	
17	2016-01-16	0	21	14	
18	2016-01-17	0	21	14	
19	2016-01-18	0	21	14	
20	2016-01-19	0	21	14	

Figure 7. Screenshot of Excel file with information about the mean number of pictures for every date and the days between upload.

A timespan for around every date of the ECF death was decided. The timespans tested for relevance were three, four and five weeks.

## 4.4. Tick count

Tick counting was performed on the cattle. OPC sprays the cattle with the anti-tick pharmaceutical Tickatraz (Amitraz) 12.5% to reduce the tick load on their cattle.

The spraying is done every 5<sup>th</sup> - 7<sup>th</sup> day. The interval has been chosen on a routine basis without any specific indicators for when to spray. Testing the interval for spraying to see is helpful to decide if it is necessary to spray every 7<sup>th</sup> day, or if the spraying interval can be prolonged or has to be reduced.

The count was arranged and carried out together with the help of the staff at OPC and took place at the different spray places. Every day a group of approximately 1500 cattle is being sprayed at one or two of the seven spray places located in different areas - Kamok, Sirrima, Morani, Muturu, Scotts, Sidai, and Lourugurugu.

Eleven groups of cattle were chosen from 11 different herds, each group containing 10 animals. Hence, in total the tick count was made on 110 different animals, two times each during a period of eight days. Ten of the herds belonged to OPC, one herd was a community herd that, it is owned by someone else but graze on the conservancy.

Herds with younger animals were selected because these were easier to handle in the corral. The primary plan was to use animals in different age categories but this was not possible. The individuals from the herds were chosen randomly.

The animals belonged to the categories steers or heifers, except for in total 14 animals. Four of these were classified as cows, three were born in 2018, and one in 2011. The remaining 10 animals belonged to a community herd and their age was unknown.

Ticks were counted in the ears and under the tail, ticks located in other areas was marked as “body”.

The first tick count on each herd was done right before the animals went into the spray race. The animals were marked with a number from 1-10. The day after the numbered animals were collected into a corral and the second tick count took place. The results were inserted into a table with the following columns:

DATE, HERD, SEX, AGE, TICK COUNT BEFORE SPRAY, EAR, TAIL, BODY, FOLLOW UP TICK COUNT, EAR, TAIL, BODY.

## 4.5. Ethical aspects

Since most of this study is done by observing animals and going through documented journals, neither animals nor people were exposed to physical or psychological harm. The only exception was recording ticks which may have caused some displeasure when the animals stood some minutes longer in the cattle corral as usual when they were handled. However, this was considered to be acceptable since the study may help developing strategies to prevent the disease, to avoid economic losses and animal suffering in the long run.

## 5. Results

We tested if the number of buffaloes correlated with the amount of pictures taken. A number of buffaloes were registered on the 32 480 pictures from 2019 and compared with the total amount of pictures. After a correlation was proven significant with the Pearson test we proceeded with comparing all pictures sorted into the buffalo folder in the years 2016-2019 against ECF deaths. The pictures are uploaded and sorted into animal-specific folders by a technician at OPC. We collected information about number of pictures per camera in the buffalo folders, and during what different time interval bunches of pictures had been taken. We calculated a mean value of pictures per date and the mean values were then compared with different timespans to the dates of ECF deaths. Data and results are presented below.

### 5.1. Daily mortality reports

In total there were 187 ECF-related deaths during the years 2016 and 2019. Out of these there were 68 heifers, 10 cows, 36 calves, 10 bulls, and 63 steers.

There was a clear overrepresentation of young animals succumbing to ECF compared to older animals. There were more cows than bulls that had died, probably because there are more cows in the herds since cows are kept longer than bulls.

*Table 1. Deaths in cattle caused by ECF per age-category and year.*

Age category	2016	2017	2018	2019
Calf	11	7	9	9
Heifer	4	10	14	40
Steer	6	10	16	31
Cow	3	4	2	1
Bull	0	1	5	4
Total	24	32	46	85

One question at issue was if there was a seasonable pattern with ECF. There was no impact of neither year nor months on the number of cows that died of ECF (Anova GLM, n.s.).

*Table 2. ECF-related deaths in cattle per month and year.*

<b>Month</b>	<b>2016</b>	<b>2017</b>	<b>2018</b>	<b>2019</b>
January	1	5	3	4
February	2	4	4	11
March	1	5	0	4
April	3	3	7	1
May	2	2	14	2
June	1	1	2	5
July	9	2	1	4
August	2	2	2	2
September	1	4	2	0
October	1	2	1	1
November	1	2	6	13
December	0	0	4	38
<b>Total</b>	24	32	46	85

## 5.2. Correlation between number of buffalo pictures and number of buffaloes on the pictures

The number of buffaloes was registered on 32 480 pictures from 2019, and compared with the total amount of pictures.

A Pearson test was done in Minitab to test if there was a correlation between the number of pictures and the number of buffaloes. In total there were 32 480 pictures sorted in Camelot, in these 2859 buffaloes were spotted. We analyzed number of buffaloes spotted in comparison to how many pictures that had been taken.

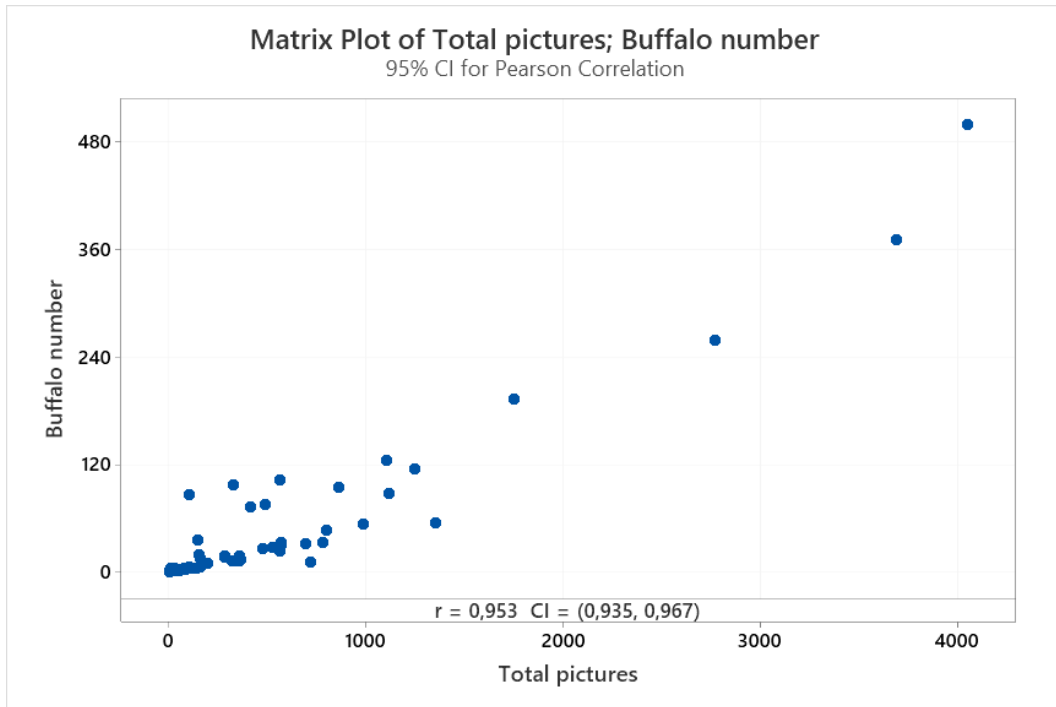


Figure 8. Pearson correlation between number of pictures and number of buffaloes.

The Pearson coefficient was 0.953 ( $p < 0.001$ ), which showed a very high correlation between pictures and buffaloes (Figure 8).

### 5.3. Number of pictures in correlation to ECF

After a correlation between number of pictures and number of buffaloes on the pictures was proven significant with the Pearson test we proceeded with comparing all the pictures that were sorted into the buffalo folder in the years 2016-2019 against the number of ECF deaths.

The pictures were uploaded and sorted into animal-specific folders by a technician at OPC. From the folders we collected and sorted information about number of pictures in the buffalo folders taken by each camera, during different download intervals. We calculated a mean value of pictures per date and the mean values were then compared with different timespans to the dates of ECF deaths. Data and results are presented below.

Since the correlation test between number of buffalo pictures and number of buffaloes showed a correlation of 0.953, the number of pictures could therefore be assumed to reflect the number of buffaloes that pass the cameras. In step two in our methods, we then compared number of pictures for all months during 2016 – 2019 with number of cattle that dies in ECF.



In total there were 153 files in the buffalo folders, which represented the number of downloads during the years. Each file contained a picture folder for each camera. The time between downloads of the pictures by the technician at the OPC varied between 3-28 days, with 7 being the most common interval. The interval of 7 days reoccurred 76 out of 153 times. 8 days was the second most common interval, this was used 14 times. The intervals were later used to calculate a mean number of pictures for each date during 2016-2019.

*Table 3. The different intervals for time passing between picture download by the technician at OPC, and times these were used.*

<b>Interval (in days)</b>	<b>Number of times the interval was used between download</b>	<b>Interval (in days)</b>	<b>Number of times the interval was used between download</b>
3	1	13	1
4	2	14	13
5	4	15	1
6	12	16	1
7	76	18	1
8	14	19	1
9	2	20	2
10	7	21	2
11	6	24	2
12	1	28	3

For year 2016 there were 32 489 pictures, in 2017; 18 008 pictures, in 2018; 36 607, and in 2019; 75 588 pictures. There was no clear connection between the pictures per year and ECF deaths per year. 2019 was a year with more ECF related deaths, and in 2019 there was also a larger number of pictures compared to the previous years, but the numbers for 2016, 2017 and 2018 does not support our theory. Due to the limited number of years it was also not possible to analyse these data statistically.

*Table 4. Pictures per year and ECF.*

<b>Year</b>	2016	2017	2018	2019
<b>Pictures</b>	32 489	18 008	36 607	75 588
<b>ECF</b>	24	32	46	85

From only pictures per year and ECF deaths per year no conclusions can be drawn regarding our hypothesis. To further examine whether there is a relation between

buffaloes and ECF deaths, we examined different timespans between death and number of pictures.

We examined if there was a significant increase in pictures prior ECF deaths for the timespans 3 weeks, 4 weeks and 5 weeks. However, there was no correlation found in the data to support this (Anova GLM, n.s.).

## 5.4. Tick count

There was an observable difference between the tick-counts and follow up tick-counts, with that being a significant decrease of ticks the day after spray. There was a larger difference between the counts with larger number of ticks. Most of the ticks the day after spray were not yet attached.

The tick count was done just before the cows went into the spray race, and the follow up-count was done the day after. For the herds Akula and Nanok the follow up-counts had to be pushed to two days after spray, because of technical difficulties with the spray race on the 1<sup>st</sup> of December, hence the follow up-count was done on the 2<sup>nd</sup> of December. The results are still in line with the following counts which could be performed according to plan. It can be assumed that Akula and Nanok would have had less than three ticks respectively the day after spray.

The results showed that there is a significant decrease in ticks per herd the day after spray ( $t=2.41$ ,  $p = 0.037$ ), see Table 6 and Table 7.

Table 5. Tick-count per herd and follow up-count day after spray.

<b>Herd name and day for count</b>	<b>Age category</b>	<b>Total tick count/herd</b>	<b>Follow up count</b>
Akula 2021-11-30	Steers	24	3 crawling
Nanok 2021-11-30	Steers and heifers	3	3 crawling
Kapiro 2021-12-01	Heifers	49	7 crawling
Cyrus 2021-12-01	Heifers	50	1 crawling
Kirimi 2021-12-01	Steers	140	17 crawling, 1 attached
Mwenda 2021-12-02	Heifers	210	19 crawling
Okoth 2021-12-03	Steers	961	9 crawling
Akriket 2021-12-06	Heifers, steers, cows*	57	3 crawling
Einuria (Community herd) 2021-12-06	Unknown	41	8 crawling
Erustas 2021-12-07	Steers	766	6 crawling, 1 attached
Mutege 2021-12-07	Heifers	403	1 crawling, 1 attached.

\*Akriket herd. Four animals counted in this herd were cows, three born in 2018, and one in 2011.

Table 6. Statistics tick count.

<b>Variable</b>	<b>N</b>	<b>N*</b>	<b>Mean</b>	<b>SE Mean</b>	<b>Minimum</b>	<b>Maximum</b>
Tick count Before spray	11	0	245.8	99.2	3.0	961.0
Tick count after Spray	11	0	7.27	1.85	1.00	19.00

Table 7. T-value and P-value.

Null hypothesis		H <sub>0</sub> : $\mu_{\text{difference}} = 0$	
Alternative hypothesis		H <sub>1</sub> : $\mu_{\text{difference}} \neq 0$	
T-Value	P-Value		
2.41	0.037		

## 6. Discussion

Our hypothesis was that increased buffalo activity would give rise to more ECF related deaths in cattle. Our results did not show any correlation between buffaloes and ECF related deaths in cattle in OPC.

OPC is an area where ECF and CD is endemic (Cook *et al.* 2021). There is a possibility that buffalo migration does not have a clear impact on ECF related deaths because there already are a diverse mixture of *T. parva* strains inside the conservancy, and new strains being brought in by buffaloes have a minimum impact on the numbers of deaths among cattle. This theory indirectly suggests that it is still the buffaloes that are causing the high prevalence of theileriosis, but it cannot be directly connected to the buffaloes' migration patterns.

The chosen method does not say whether there are any new buffaloes coming in from other areas, it only maps activity along the corridors, and it does not take buffalo movement inside the reserve into consideration. Buffalo activity around the corridors did not affect the ECF-related deaths but the result cannot exclude that buffalo migration into the reserve, or buffalo movement inside the area, had an impact on ECF prevalence.

This study does also lack data on how many cattle that have survived infection and have been treated during the years. We therefore suspect that our method is not sensitive enough, and that buffalo migration still might have an impact on ECF prevalence in OPC's cattle.

Different factors that could be improved with the method used in this study and complementary methods are discussed below, together with confounding factors and a discussion about preventive measures around ticks and immunization.

### 6.1. Does buffalo activity on camera reflect the population inside the reserve?

The cameras are situated at the corridors where all animals except rhinos can pass in and out of the OPC.

For sorting pictures and counting buffaloes, the initial thought was that it would be possible to determine whether the buffaloes were walking in or out of the conservancy in pictures, but for the majority there was not a clear direction. Either

a direction of in or out could not be decided from the image, or the animals were simply grazing along the corridors. This makes it hard to estimate any number of animals migrating in or out.

Counting buffaloes in the pictures can only give us an estimation of buffaloes inside the game reserve. It is not possible to count every buffalo passing the cameras since the cameras can miss buffaloes moving around the cameras because of darkness or distance. In contrast, sometimes individuals could be counted twice on pictures taken by the same camera, or taken by a different camera in the same or different corridor. There are also problems such as blind spots in corridor 1. Pictures taken by camera 1D were not included in this study because of poor quality, there was also less pictures in the folder 1D compared to other folders. Another unknown factor is the number of buffaloes inside the OPC before the start of our study.

The cameras can despite this still give an indication of whether there are more buffaloes inside the reserve during a certain period, by determining if there was more buffalo activity along the corridors. The assumption was that buffaloes along the corridors can be assumed to reflect the number of buffaloes inside the conservancy. It is hard to evaluate the accuracy of this method though. The OPC covers a big area and it is unknown if the activity along the corridors tells anything about the direct or indirect contact between cattle and buffaloes in all the zones in the reserve. A positive correlation during the four years could however have suggested that increased buffalo activity along the fence also means increased contact and transmission of ECF between buffaloes and cattle.

Time was a limiting factor, therefore not all pictures from the years 2016-2019 were sorted in Camelot in the way the 32 480 pictures from 2019 were. The pictures from year 2019 were chosen to be analysed because in the daily mortality reports (DMRs) from the OPC there was clear spike in ECF-deaths during December 2019.

The results from the buffalo-count in Camelot was instead used to decide if there was a correlation between number of pictures and number of buffaloes. A correlation was previously not obvious between these two factors since there was a risk that the reason for increased pictures could be that there are many pictures of the same individuals, especially when grazing or resting close to the cameras. However, we found a strong Pearson correlation between number of pictures and number of buffaloes, which is a good indicator for showing that more pictures actually indicate more buffaloes present.

Moving forward to step two in our method with using only number of pictures to further analyse any correlation between ECF-deaths and buffalo activity, this method would be improved with a constant time interval for picture download. The time intervals differed between the picture downloads, and the time between downloads could have affected the mean number of pictures per date. Since most intervals are in similar timespans, we still considered the data to give sufficient information for our purpose.

A small number of pictures sorted in Camelot revealed that some pictures are sorted into the wrong folder, approximately 2%. Other species spotted in the buffalo-folder were elephant, gazelle, hyena, zebra and lion. Some images contained cars or were just dark. In step two in the method where number of pictures are just looked at per folder, and not the pictures themselves, it is not possible to know how many of these pictures that are incorrectly placed in the folder. On the other hand is it also likely that some buffalo pictures were missing since they were sorted into other species' folders.

Sorting and counting every buffalo in the pictures would give a more certain result by giving specific dates and a more representative number of buffaloes, it would also exclude wrongly sorted pictures. It was hard deciding a direction from the pictures though, cameras taking pictures from another angle could possibly have helped in deciding a direction. Knowing the direction would be a better tool for estimating the buffalo population inside the conservancy.

## 6.2. The impact of rainfall

There are many contributing factors that can give rise to the spike in ECF cases in relation to the host-pathogen-environmental triangle. Rainfall is a confounding factor and affects both buffalo migration and tick numbers.

Access to water affects buffaloes' migration patterns, their choice of habitat and herd size (Naidoo *et al.* 2012; Megaze *et al.* 2013; Roug *et al.* 2020). This in turn is affected by weather, which has an impact on different types of forage (Winnie Jr. *et al.* 2008; Cornelis *et al.* 2014; Bennitt *et al.* 2016). Kenya typically has two rain seasons; one during March to May with so called long rains, and another rainy period in October and December with short rains, with local exceptions (Ongoma 2019).

Migrating buffaloes move away from their stational water sources to temporal ones during wet seasons (Megaze *et al.* 2013; Roug *et al.* 2020). During the dry seasons more buffaloes stay in areas close to a permanent water source, and often their home ranges are bigger compared to wet seasons (Naidoo *et al.* 2012; Megaze *et al.* 2013; Roug *et al.* 2020).

With more rainfall within OPC, there is probably a higher likelihood for buffaloes wanting to stay inside the conservancy since wetter periods improve amount, and quality of forage (Bennitt *et al.* 2016). Herd sizes are also usually bigger during wet seasons, and their home ranges smaller (Naidoo *et al.* 2012; Megaze *et al.* 2013; Cornelis *et al.* 2014; Roug *et al.* 2020). During wet seasons it is therefore likely that OPC has higher densities of buffalo.

Rainfall also greatly increases tick-numbers (Keesing *et al.* 2018; Chepkwony *et al.* 2020), and since ECF is reliant on the number of ticks and their life cycle, rainfall should also have an impact on ECF related mortalities. The perception by the staff

at the OPC is that ECF prevalence goes up around three weeks after rainfall. ECF related deaths have been seen to have a correlation with rainfall in a study done on OPC by Chepkwony *et al.* (2017). Rainfall can therefore increase ECF related deaths both by increasing the number of ticks, and the number of buffaloes.

Our result does not show a correlation between buffalo activity around the corridors and ECF mortality. However, because of previous research we still suspect that there could be a liaison, but our method might not have been sensitive enough. The variations in OPC's buffalo population and their potential connections to ECF, should proposedly be examined in correlation to rainfall to further understand causing factors.

According to Richard van Aardt, OPC's buffalo numbers have increased over the years. It would be interesting to follow herds of buffalo in the area to see if there are any resident herds within the conservancy or returning migrating herds. Roug *et al.* (2020) found that buffaloes returned to their drier home habitat during drought. OPC provides man-made stational drinking sources for the animals inside the reserve. Buffaloes may therefore choose to make OPC their stationary home during dry seasons, or their returning home range and that is why the numbers have gone up. The aspect of competition between ungulate species has not been examined for this study but may also influence buffaloes' migration in and out of the reserve. Regulation of the population within the conservancy should further be examined.

Rainfall affects vegetation. Factors related to vegetation such as grass height was not examined for this study but could potentially affect both tick densities and forage behavior of both buffaloes and cattle.

### 6.3. Tick count

It is important to emphasize that the tick count was primarily done to evaluate the interval for which OPC spray their cattle, and not the drug substance Amitraz.

Our results showed that the cattle collected a notable number of ticks during the days between spray. The interval for spraying could therefore be considered necessary to keep the tick load down.

During the period for when the tick count was done there had been more rainfall than usual in the area which is important to take into consideration when deciding the spray interval. In OPC's experience more rainfall brings more ticks, and later more ECF cases, therefore they often shorten the spray interval to 5 days during wetter periods. OPC's experience seems to agree with previous research where rainfall has been shown to increase tick numbers and tick activity in the life stages nymph and adult (Keesing *et al.* 2018; Chepkwony *et al.* 2020). Another factor that can affect the number of ticks on the cattle is when the acaricide is washed off by rains.

From our trial we cannot exclude that the interval can be prolonged during other weather conditions, especially during drier periods. During dry seasons the interval should be tested again in a similar manner. If tick load appears to be lighter, proposedly a small number of animals from each herd could be relieved from spraying with follow-up counts daily, to help decide a dry season spraying interval.

The tick count should have been done on different age categories since it could give an indication of whether there is a difference in tick load between the different age groups. Even if done in this trial, it would have been hard to evaluate an age difference in this short amount of time, since herds graze in different areas where the exposure to ticks may differ. Tick counts need to be repeated several times due to changes in surrounding conditions to be able to give reliable results.

To improve efficacy for the trial and decrease the time for cattle in the corral, a scale for specifically the ears can be decided where each scale represents an interval for the amount of ticks, instead of counting every tick in the ear. For example a scale of 0-6 where 0 represents, no ticks, scale 1; one to five ticks, 2; five to 10 ticks, 3; 10-20 ticks, 4; 20-50 ticks, 5; 50-100 ticks, and 6; 100 + ticks.

## 6.4. Other prophylactic measures at the OPC

ITM (Infection and Treatment Method) vaccination has been proven to be an effective way to protect sensitive breeds of cattle against ECF in earlier research, but in areas where cattle graze close to wildlife protection has been unsuccessful (Bishop *et al.* 2015; Sitt *et al.* 2015). This is the case on OPC, which also conducted a study on the Muguga cocktail with the International Livestock Research Institute (ILRI) where they could not see a difference in survival between the vaccinated group and the control group (Sitt *et al.* 2015; van Aardt 2021).

OPC has tried different strategies to decrease the impact of ECF and the conservancy is trying another method to build immunity in their animals. They expose their calves at the age of three months to a heavy tick load, by not treating them with anti-tick treatment. They put the calves on a pasture, often where buffaloes have been grazing. After two weeks they check on the animals and do a tick count to evaluate the tick challenge. Treatment with both short term (day one) and long acting oxytetracycline (day two) is then started, regardless of if the animals are showing symptoms or not.

The hope for routine is to immunize the cattle with local *T. parva* strains and the perception is that it works well and that they have fewer ECF related deaths in these animals. The method is per definition an ITM and theoretically it should work in the same way the ITM vaccination with the Muguga cocktail does, but it needs to be tested.

One aspect contradicting the theory is that buffalo strains seem to fail to circulate within cattle populations (Magulu *et al.* 2019; Allan *et al.* 2021). Cattle surviving a



*T. parva* infection of cattle-derived *T. parva* become carriers of the parasite and can circulate within the cattle population. This enables the cattle to eventually build up an immunity to those strains and the severity of infection decreases. Buffalo strains do not seem to be shared between cattle though, even in cattle living close to wildlife (Bishop *et al.* 2015; Sitt *et al.* 2015; Allan *et al.* 2021), which points to a limited immunity build-up against buffalo-derived *T. parva*.

There is a hypothesis that *T. parva* circulating in cattle is a subpopulation that has developed to be able to circulate within cattle (Morrison *et al.* 2020). Morrison *et al.* (2020) mention several earlier studies in their review where there have been trials on altering buffalo-strains to act more like cattle-strains. Overall, these have been unsuccessful, but there are a few exceptions. Maritim *et al.* (1992) managed to alter a buffalo *T. parva* strain to behave like a cattle strain. After five tick/cattle passages the number of schizont parasites and piroplasms were high enough to become infectious to ticks (Maritim *et al.* 1992) and passed on to cattle. There is a possibility that OPC's routine aids in altering a buffalo-strain to act as a cattle-strain, which now circulates within their cattle population. If so, their natural vaccination routine should work well.

A controlled natural exposure – treatment routine that simulates the Muguga ITM vaccination but with local strains, could give the correct immunity response. Natural exposure to ticks is difficult to control though and results will not be homogenous among groups, mixed infections with different strains are documented (Bishop *et al.* 2015; Allan *et al.* 2021). Most likely the groups of calves are exposed to different buffalo strains and a cross-protection mechanism would be necessary for natural vaccination to work well.

Cross-protection has been seen between different cattle-derived *T. parva*, through the gene coding for p67 (Nene *et al.* 1996). Bishop *et al.* (2015) also observed potential cross-reactivity in monoclonal antibodies between buffalo- and cattle-derived strains. However, they also found vaccination with the commonly used cattle strains to fail in giving protection to several buffalo strains.

Cross-immunity therefore seem to exist but is limited between the different strains. By doing OPC's calf routine perhaps the desired cross-protection can be reached by the use of local strains and “kick-start” the calves' immune system in a relatively controlled manner. Their immunity could theoretically then continue to evolve under the continuous exposure the different *T. parva* infected ticks over the span of their lifetime.

It would be extremely interesting to evaluate the routine by comparing immune response in one group of calves that are exposed to ticks in the previously described manner, with a control group. It would also be helpful to investigate the parasite strains on the conservancy. This would give further useful information to the research regarding *T. parva* and the immune response and cross-reactivity between buffalo strains and cattle strains.

## 6.5. Discussions around future methodology

A complementary method that was planned to do during the field study to investigate herd immunity in OPC's cattle was to sample a number of cattle for antibodies against ECF. The suggestion was to sample blood from the moment of slaughter to avoid unnecessary suffering by restraining and taking blood samples on live animals. On site this was harder than expected and many factors contributed to that the idea had to be cancelled. A serology screening for ECF could potentially have given an indication for how big of a portion of the animals that carry some sort of antibody against ECF. The cattle are marked in the ears and the markings tell the birth year and month of the animal, which in turn gives information about the likelihood of the animal having gone through OPC's calf routine or not. This could later have been discussed with the result of ECF related deaths and buffalo activity. We wanted to map antibodies in OPC's own animals, since previous studies by ILRI mainly been done on animals from outside the conservancy, owned by ILRI. Doing this study in future would increase knowledge about ECF.

Another method that was planned was transect drives. The transect drives were supposed to help understand buffalo movement inside the reserve by mapping number of buffaloes in certain areas, and potentially give information about if cattle and buffaloes share pasture grounds. The transect drives had to be canceled because of financial restrains. Other suggestions for mapping buffalo movement could be pictures taken in air by drones or flights. Counting cattle and buffaloes directly and not only at the corridors will help to get a better picture of how much cattle and buffaloes overlap.

There was no clear data on how many ECF cases there were in total for the years in this study, only the deaths were registered. There was a relatively small number of deaths which makes it harder to perform a correct analysis. OPC are used to ECF and are well aware of the signs and when to treat, this study therefore misses a big number of ECF cases to compare against buffalo activity. All cases of ECF regardless of outcome would give more certain results.

## 7. Conclusions

### 7.1. Correlation between buffalo activity and ECF related deaths

A correlation between pictures of buffaloes around the corridors and ECF-related deaths in OPC's cattle was not found in this study. However, a possible liaison is not yet excluded though. The method used is not sensitive and is limited in those ways that it only examines a minor area and only maps activity, not direction of which the animals are moving. It does not give any information about the buffalo population and their movements inside the reserve. Further research on buffalo movement in and out of the reserve and any direct or indirect contact with cattle should be mapped to be able to say if buffalo movement affects ECF related deaths inside OPC. This should include any seasonal patterns in buffalo migration and in ECF prevalence. This would be a useful contribution to the research of ECF and CD to help understand the role that buffaloes play in affecting the clinical severeness of disease and what preventative measures that can be used in cattle management.

### 7.2. Tick count

For this study the conclusion from the tick-count is that the interval used during the time for our trial was required. However, a longer study period under different weather conditions should be performed to be able to evaluate spray intervals for different weather conditions. There is a chance that the interval could be prolonged during drier circumstances, thus reducing costs for prophylactic measures, and reduce environmental impact.

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## Acknowledgements

I would like to give thanks to all of the workers at OPC, and a special thanks to Charles Mathenge and Richard van Aardt for their incredible support and motivation for our project.



# Populärvetenskaplig sammanfattning

East Coast Fever (ECF) är en parasitsjukdom som förekommer i 11 afrikanska länder i framförallt östra och centrala Afrika. Den dödar ca 1 miljon kor varje år och beräknas bidra till ekonomiska förluster på över 300 miljoner amerikanska dollar årligen.

ECF orsakas av parasiten *Theileria parva*, en encellig organism (protozo) som sprids med den bruna öronfästingen. Parasiten angriper immunförsvaret och kor som drabbas får svullna lymfknutor, feber, försämrat allmäntillstånd och svårare att andas. Djur som överlever infektion blir permanent infekterade och fortsätter bära, och sprida parasiten.

Dödligheten varierar med flera olika faktorer. Den varierar bland annat mellan koraser, och är framför allt hög i raser av nötkreatur som är mer nyintroducerade till den afrikanska kontinenten, hos dessa är dödligheten ofta 100 % om korna går obehandlade. Afrikanska bufflar (och vissa inhemska ko-raser) blir inte sjuka av parasiten och fungerar som tysta smittbärare av sjukdomen.

Parasiten *Theileria parva* delas upp i olika stammar. I stort delas in de in i två grupper - stammar som härstammar från kor och stammar från bufflar. Parasitstammar som sprids från bufflar till kor är farligare än de som cirkulerar bland boskapen. Detta tror man har att göra med att kor kan till en viss del bygga upp en immunitet mot de parasitstammar som cirkulerar mellan kor inom ett område, medan de varianter av parasiter som kommer från bufflarna varierar mer i sitt utseende. Parasitstammar blandas hos bufflar när parasiten förökar sig, således utvecklas hela tiden nya stammar som bufflarna kan bära med sig och sprida till nya områden. Kor som går på beten där många bufflar också rör sig riskerar därför att ständigt utsättas för nya parasitstammar som de inte har någon immunitet mot. Buffelformen av ECF kallas ibland för Corridor disease (CD).

ECF behandlas ofta med antibiotika, men det finns även sätt att försöka förebygga sjukdom. Djuren kan skyddas med hjälp av fästingmedel som appliceras på djurens hud med jämna mellanrum. Det finns också vaccin, den vanligaste typen kallas *Muguga Cocktail*. Vaccinet är ett s.k. kallat levande vaccin där en mix av stammar av parasiten injiceras i kon som sedan behandlas med antibiotika. Vaccinet har funnits sen 70-talet och verkar kunna ge skydd mot sjukdom, men det finns också flera problem med vaccinet. Levande vaccin är svårare att kontrollera och korna riskerar att utveckla en livshotande infektion eftersom parasitstammarna är livsdugliga, därför behandlar man också djuren med antibiotika efteråt. Detta bidrar

till en överdriven antibiotikaanvändning. Det är också svårt att förutse effekten av vaccinet. I områden där kor rör sig i områden med många bufflar så verkar vaccinet ha dålig effekt.

Denna studie utfördes på, och i samarbete med på Ol Pejeta Conservancy (OPC) i Laikipia County i centrala Kenya. OPC är ett stort stängslat reservat som arbetar för att bevara vilda djur, med fokus på noshörningar. OPC äger också ca 6000 kor av rasen Boran, och deras tamboskap betar tillsammans med vilda djur inklusive bufflar, i ett så kallat ”*Livestock and wildlife integrated system*”. OPC har stora problem med ECF trots att de aktivt jobbar med rutiner för att förhindra sjukdomen. De använder framför allt fästingmedel men de har också utfört studier med *Muguga*-vaccinet, dock har detta inte fungerat för dem.

I denna studie undersöktes det om det kunde finnas ett samband mellan ökad buffelaktivitet på OPC och ökat antal dödsfall i ECF hos deras kor.

Buffelaktiviteten på området uppskattades genom att titta på bilder från infra-röda kameror som övervakar s.k. korridorer, vilka är de enda passagerna på OPC där vilt kan passera ut och in. Aktiviteten från bilderna jämfördes med information från journaler där OPC registrerar alla dödsfall per dag (av olika orsaker) hos deras kor. Registrerade antal ECF-dödsfall under åren 2016-2019 jämfördes med data som samlats in från buffelaktivitet motsvarande år. För att ta hänsyn till tid innan insjuknande och dödsfall jämfördes buffelaktiviteten tre, fyra och fem veckor innan ett dödsfall.

Resultaten från studien visade inget samband mellan registrerad ökad buffelaktivitet och dödsfall i ECF, men ett samband anses inte kunna uteslutas. Metoderna för studien misstänks inte vara känsliga nog för att hitta ett möjligt samband.

Korridorerna är ett litet område som övervakas av kamerorna och det ger därför begränsat information om populationen bufflar och deras rörelser inne på området. Det gick heller inte att registrera vilket håll bufflarna rörde sig utan endast aktiviteten. Det behövs därför mer detaljerade studier där bufflarnas rörelser inom området, och rörelser i relation till kornas undersöks.

I studien fanns endast tillgång till antalet dödsfall för ECF, och inte totalantalet ECF-fall, vilka bör inkluderas för en korrekt bild. Bufflarnas rörelser bör också undersökas i relation till säsong och nederbörd, då nederbörd påverkar både bufflars migration och fall av ECF.

I denna studie så testades också intervallet för när OPC sprayar sina kor med fästingmedel. Detta är en omständlig rutin där ca 1500 kor sprayas varje gång genom att fösa dem genom fållor som leder dem genom ”spray-duschar” som drivs av motorer. OPC sprayar på rutin korna 1 gång i veckan men intervallet tror dem skulle kunna förlängas i vissa perioder, och på så sätt undvika onödiga kostnader och minska deras användning av fästingmedel.

Fästingar räknades i kornas öron på totalt 110 kor precis innan de skulle gå in i spray-fällan, och dagen efter att de blivit behandlade. Majoriteten av djur samlade på sig stora antal fästingar, varav vissa hade över 100 fästingar i öronen. Spray-intervallet som användes under tiden för studien ansågs därför nödvändigt under de omständigheter som rådde. Under perioden var det mer nederbörd än vanligt på området. Intervallet bör testas under flera olika väderomständigheter för att se om intervallet kan förlängas under torrare perioder. OPC upplever att det förekommer mindre fästingar under torrare förhållanden och detta stöds även av annan forskning.

Genom att bättre förstå hur bufflar och deras rörelsemönster påverkar fall av ECF, desto lättare blir det att ta fram strategier för att förhindra sjukdom. Mer forskning behövs därför på området.

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