The Impact of Bluetongue on Rumminants Mortality. (Bovine and Ovine)

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CERTIFICATION

This is to certify that this research work was carried out by **NZUMENANG NZUONKWELLE** under our supervision.

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DEDICATION

This work is dedicated to my family.

ABSTRACT

Bluetongue is a disease of sheep, but cattle are the principal vertebrate reservoirs of the virus. Once established, “it is impossible to actively eradicate bluetongue virus”. The virus will circulate, generally subclinically, in cattle and other ruminants, and in midges.

The objective of this study was to examine the correlation between the bluetongue incidence data (2006) and the mortality data (2006). To achieve the main objective of this report, the difference in the 2006 mortality and mean mortality of previous year’s (2002 to 2005) was obtained. This difference was used to examine the correlation of the Bluetongue incidence and the mortality in 2006. The generalized linear mixed model with negative binomial as distribution and the log link as the link function were used in analyzing the data. The result of the analysis revealed that there was a weak correlation between the mortality and incidence in 2006. Thus the result of the study seem to show that Bluetongue had no influence on ruminant mortality during the year 2006. There was a difference in mortality and incidence in the various provinces. There is no difference of incidence between species, as well as mortality.

Key Words: Generalized linear mixed model, Negative binomial. Bluetongue (BT) Virus

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TABLE OF CONTENTS

LIST OF TABLES AND FIGURES

1 INTRODUCTION

1.1 Background knowledge

Bluetongue (also called catarrhal fever) is an infectious, noncontagious arthropodborne viral disease primarily of domestic and wild ruminants. Infection with bluetongue virus is common worldwide but is usually subclinical or mild in most infected ruminants. Bluetongue is almost exclusively a disease of sheep, particularly the fine-wool and mutton breeds, and less frequently of cattle, goats, buffalo, deer, dromedaries and antelope. There are no reports of human transmission [7][25].

The virus was thought to be confined to Africa but in the past 50 years Bluetongue has increasingly been recognized wherever substantial populations of ruminants occur in the tropics and subtropics. The initial detection of virus in countries outside Africa has sometimes occurred because of spectacular outbreaks of disease. Recent outbreaks of Bluetongues in the Mediterranean Basin have followed this pattern with severe losses in sheep [8][11][24].

It appears that at least some serotypes of Bluetongue disease may now be enzootic in parts of southeastern Europe. Bluetongue has been observed in Australia, the USA, Africa, the Middle East, Asia and Europe[2]. Its occurrence is seasonal in the affected Mediterranean countries, subsiding when temperatures drop. It has been spreading northward since October 1998, perhaps as a result of global warming [20]. In August 2006 cases of bluetongue were found in the Netherlands, then Belgium, Germany, and Luxembourg [6][26].

Bluetongue virus is transmitted biologically by *Culicoides* insects (biting midges), but only a limited number of species are efficient vectors. Cattles are the main amplifying hosts for Bluetongue virus. They are also probably important maintenance hosts. The competent *Culicoides* vector species feed more abundantly on cattle.

The virus cannot be transmitted between susceptible animals without the presence of insect carriers. The incidence and geographical distribution of bluetongue depends on seasonal conditions, the presence of insect vectors, and the availability of the susceptible species of

animals. The insect carriers, biting midges, prefer warm, moist conditions and are in their greatest numbers and most active after it rains. Bluetongue virus does not survive outside the insect vectors or susceptible hosts. Animal carcasses and products such as meat and wool are not a method of spread. Survival of the virus within a location is dependent on whether the vector can overcome winter in that area [20][24]. Distribution throughout the world parallels the spatial and temporal distribution of vector species of *Culicoides* biting midges, which are the only significant natural transmitters of the virus. Of more than 1,400 *Culicoides* species worldwide, fewer than 20 are actual or possible vectors of bluetongue virus.

All ruminants, including sheep, goats, cattle, buffaloes, camels, antelopes and deer, are susceptible to Bluetongue virus infection. Of the domestic species, sheep are clinically the most severely affected. Sickness is sometimes reported in goats and severe disease and mortalities occur in white-tailed deer in the United States. Although the infection of cattle is of great epidemiological significance, it is generally sub-clinical. Horses and pigs are not infected by Bluetongues virus but *Culicoides* may feed upon them and the premises where they are kept may provide suitable vector breeding sites [24].

Major signs are high fever, excessive salivation, swelling of the face and tongue and cyanosis of the tongue. Swelling of the lips and tongue gives the tongue its typical blue appearance, though this sign is confined to a minority of the animals. Although the tongues of human patients with some types of heart disease may be blue, this sign is not related to bluetongue disease [18] [25].
Recovery is very slow. The incubation period is 5–20 days, and all signs usually develop within one month. The mortality rate is normally low, but is high in susceptible breeds of sheep. In cattle and wild ruminants infection is usually asymptomatic despite high virus levels in blood.
Deaths may occur at any stage up to a month or more after the onset of signs. The course of the disease in sheep can vary from peracute to chronic, with a mortality rate of 2-30%. Peracute cases die within 7-9 days of infection, mostly as a result of severe pulmonary edema leading to dyspnea, frothing from the nostrils, and death by asphyxiation. In chronic cases, sheep may die 3-5 wk after infection, mainly as a result of bacterial complications, especially pasteurellosis, and exhaustion [24]. Mild cases usually recover rapidly and completely. The major production losses include deaths, unthriftiness during prolonged convalescence, wool breaks, and possibly

reproductive loss. Infection of pregnant ewes may lead to abortions, mummified foetuses, or the birth of stillborn or weak lambs, which may have congenital defects. Goats are less commonly, and less severely, affected than sheep. The pathogenesis is similar and the clinical signs are milder.

Infection in cattle, although of great epidemiological significance, is generally sub-clinical. A report from the United States suggested only 0.01% of cattle infected with Bluetongue virus show clinical signs. These include inflammation and mucosal erosions in the mouth and nose, mild laminitis and a stiff gait. Susceptible cattle and sheep infected during pregnancy may abort or deliver malformed calves or lambs. The malformations include hydranencephaly or porencephaly, which results in ataxia and blindness at birth.

The typical clinical signs of bluetongue enable a presumptive diagnosis, especially in areas where the disease is endemic. Suspicion is confirmed by the presence of petechiae, ecchymoses, or hemorrhages in the wall of the base of the pulmonary artery and focal necrosis of the papillary muscle of the left ventricle. These highly characteristic lesions are usually obvious in severe clinical infections but may be barely visible in mild or convalescent cases. In many areas of the world, bluetongue in sheep, and especially in other ruminants, is subclinical and, therefore, laboratory confirmation based on virus isolation in embryonated chicken eggs, susceptible sheep, or cell cultures, or the identification of viral RNA by PCR is necessary[19][25].

Bluetongue can be a costly infection for several reasons. The clinical disease in sheep can be severe, resulting in deaths, weight loss and wool break. Bluetongue is a disease of sheep, but cattle are the principal vertebrate reservoirs of the virus. Once established, it is impossible to actively eradicate bluetongue virus [24]. There is need for some preventive measures.

There is no efficient treatment. Prevention is via quarantine and movement controls to prevent spread. Immunization of sheep remains the most effective and practical control measure against bluetongue in endemic regions [19][23]. Also husbandry procedures to control vectors, reduce transmission and protect susceptible animals. Another means of prevention is by tracing and

surveillance to determine the extent of virus and vector distribution, Zoning to define infected and disease-free areas, including the inspection of aircraft.

1.2 Objective of the study

The main objective of this study is to examine the correlation between the bluetongue incidence in 2006 data (base on the sampling dates) and the 2006 mortality data (rendac rendering plant) while correcting for the effect of species and provinces.

1.3 Organization of the report

Section 1 of this report provided a brief introduction on bluetongue disease. The data description is introduced in Section 2. Section 3 presents the statistical methodology used to investigate the objective of the study. The Results of the statistical analysis are presented in Section 4. Section 5 holds the discussion and conclusion and Section 6 has the recommendation, which is then followed by the references and the appendix.

2 DATA DESCRIPTION

Three data sets were used in this study; the mortality data for cattle which were recorded from the year 2002 to 2007 as well as for sheep and goat called the sheep mortality, and the dataset of the Outbreak of the bluetongue cases recorded in the year 2006 called incidence data. The mortality data set were measured as the number animals that died while the incidence data was measured as the number of herds infected by Bluetongue.

The data sets for cattle and sheep mortality were transformed from mortality per day to mortality per week by summing the number of death per day for a given week, as were as for the incidence data. The calendar weeks (1-53) were used for easy interpretation and such that the mortality can be studied for each season, same as for the incidence.

In order to analyze these data sets certain assumptions were made; the previous year's mortality is mortality not due to Bluetongue incidence and is constant overtime. Also it is known that Bluetongue never occurred before 2006. These assumptions are plausible to better estimate for Bluetongue mortality in 2006.

To achieve the main objective of this report, the difference in the 2006 mortality and mean mortality of previous year's (2002 to 2005) was obtained. The assumption is that this difference is the mortality due to Bluetongue. The data of the Bluetongue incidence was then merged with the difference in mortality obtained for species Bovine and Ovine. This difference was used to examine the correlation of the Bluetongue and the mortality in 2006. If this correlation is strong (weak) or high then the outbreak of bluetongue occurred in 2006.

As an illustration, Table 1 shows the initial data when incidence and mortality were merged for species Bovine. Table 1 shows Part of the data for Bovine for the first 24 observations for the province of Limburg. The variables *INC* and *MORT* are incidence and mortality. The outbreak of Bluetongue (*INC*) in 2006 was first observed in week 33 in Belgium and it was also observed in week 33 in Limbourg. The data was re-arranged such that the time of incidence should match the time of death.

Table 1: Data for Bluetongue Mortality and Incidence

	WEEKS	province	INC	MORT	YEAR
665	31	LIMBOURG	.	0	2006
666	32	LIMBOURG	.	54	2006
667	33	LIMBOURG	1	0	2006
668	34	LIMBOURG	2	4	2006
669	35	LIMBOURG	2	12	2006
670	36	LIMBOURG	2	11	2006
671	37	LIMBOURG	.	1	2006
672	38	LIMBOURG	.	60	2006
673	39	LIMBOURG	3	33	2006
674	40	LIMBOURG	3	33	2006
675	41	LIMBOURG	5	41	2006
676	42	LIMBOURG	3	31	2006
677	43	LIMBOURG	4	28	2006
678	44	LIMBOURG	.	25	2006
679	45	LIMBOURG	.	45	2006
680	46	LIMBOURG	2	42	2006
681	47	LIMBOURG	.	43	2006
682	48	LIMBOURG	1	0	2006
683	49	LIMBOURG	.	17	2006
684	50	LIMBOURG	.	0	2006
685	51	LIMBOURG	.	0	2006
686	52	LIMBOURG	.	0	2006
687	53	LIMBOURG	.	0	2006
688	54	LIMBOURG	.	65	2007

This is because when an animal is diagnosed with bluetongue it may take several days before the animal dies.

The procedure “Expand” in SAS and “transform=lead’ was used in achieving this goal. Each lead time obtained was used to fit a Generalized Linear Mixed Model with negative binomial as distribution. The response variable was *Mort* (mortality) model against *INC* until when the lead time was found to be significant. The AIC criterion was used to compare different lead times that were significant. Table 8 in the appendix shows the different lead times that were significant and their AIC values. The assumption is that, this is the time it takes an animal to die after it is diagnosed with Bluetongue disease. Base on the data for the Bovine species, after an animal is diagnosed with Bluetongue it will take 11 weeks after which the animal would die based on the criteria of AIC that smaller value is better. However 1 week was considered appropriate as it is known from literature that it may take 1 week for the animal to die [4]. So 1 week was

considered despite the AIC value was not the smallest. The incidence and corresponding mortality after adjusting for the time (1 week) to death after diagnosis is shown in table 2.

Table 2: The lead time for the Bovine

	WEEKS	province	INC	MORT	MORTALITY	Year
348	31	LIMBOURG	.	0	54	2006
349	32	LIMBOURG	.	54	0	2006
350	33	LIMBOURG	1	0	4	2006
351	34	LIMBOURG	2	4	12	2006
352	35	LIMBOURG	2	12	11	2006
353	36	LIMBOURG	2	11	1	2006
354	37	LIMBOURG	.	1	60	2006
355	38	LIMBOURG	.	60	33	2006
356	39	LIMBOURG	3	33	33	2006
357	40	LIMBOURG	3	33	41	2006
358	41	LIMBOURG	5	41	31	2006
359	42	LIMBOURG	3	31	28	2006
360	43	LIMBOURG	4	28	25	2006
361	44	LIMBOURG	.	25	45	2006
362	45	LIMBOURG	.	45	42	2006
363	46	LIMBOURG	2	42	43	2006
364	47	LIMBOURG	.	43	0	2006
365	48	LIMBOURG	1	0	17	2006
366	49	LIMBOURG	.	17	0	2006
367	50	LIMBOURG	.	0	0	2006
368	51	LIMBOURG	.	0	0	2006
369	52	LIMBOURG	.	0	0	2006
370	53	LIMBOURG	.	0	65	2006

The same procedure was applied to the Ovine data and the lead time was 8 weeks. Also 1 week was considered same as Bovine. The 1 week lead time that was used for the analysis of the data. The fact that the average time it takes the disease to kill the animal in different species may be different, motivated the species analysis for determining the “lead” time in weeks. To properly achieve our objective of the ”lead” time the mortality data of 2007 was used. For example if an animal was diagnosed in December for bluetongue, it may die after 1 week for either species which is the next year (2007).

The data sets for Bovine and Ovine were then merged. The final data set was arranged in a multivariate format as special case of the bivariate format with weeks as cluster. The incidence and mortality were clustered in weeks. The final data had 2330 observations and 5 variables with the response variable as *Respvalue* , covariates included were *species, province* , *Resp* and the variable *weeks* was the subject. The Variable *Province* had 11 levels which are the provinces of Belgium; *Species* had 2 levels Bovine and Ovine. *Resp* had 2 levels representing Mortality and Bluetongue Incidence. The *Respvalue* is the observed value for Mortality and Bluetongue Incidence. The variables used in the analysis of the mortality and Incidence data are described in Table 3.

Table 3: Variable description for the data used in the analysis

Variable	Description	Variable
Weeks	Clusters of mortality and incidence which ranges 1 to 53.	Discrete
Resp	Category for mortality and incidence	Discrete
Respvalue	Values for Resp.	Continuous
Province	11 Provinces of Belgium	Nominal
Species	Bovine and Ovine	Nominal

3 STATISTICAL METHODOLOGY

This section presents a description of the methods that will be used throughout the Thesis.

3.1 Exploratory Data Analysis

Exploratory data analysis (EDA) was performed to bring out the various relationships, structures and patterns or trends in the data. Quantitative techniques were used to obtain summary statistics such as mean, variance of response variable. Histograms and Scatter plot were used to depict patterns of the response variables.

3.2 Generalized Linear Mixed Model (GLMM)

In cluster data, measurements taken repeatedly on the same cluster (weeks) tend to be correlated hence appropriate statistical methodology must be applied to take into account this correlation. The generalized linear mixed model (GLMM) was used to account for this correlation between the bluetongue incidence in 2006 and the mortality in 2006 on the same week. The general model formulation is given as [13][14]

Let Y_{ij}, be the jth outcome measure for subject(Weeks) $i, i = 1,...,N$, $j = 1,...,n_i$ and Y_i is the n_i-dimensional vector of all measurements available for the subject i. It is assume that, conditionally on q-dimensional random effects b_i, assumed to be drawn independently from the $N(0, D)$, the outcomes Y_{ij} are independent with densities of the form

$$f_i(y_{ij} \setminus b_i, \beta, \phi) = \exp\left\{\phi^{-1}\left[y_{ij}\theta_{ij} - \psi(\theta_{ij})\right]\right\} + c(y_{ij}, \theta), \text{ with}$$

$$\eta(\mu_{ij}) = \eta\left[\mathrm{E}(Y_{ij} \setminus b_i)\right] = x'_{ij}\beta + z'_{ij}b_i$$

for a known link function $\eta(.)$,with x_{ij} and z_{ij} p-dimensional and q-dimensional vector of unknown fixed regression coefficients, and with ϕ a scale parameter. Finally, let $f(b_i \setminus D)$ be the density of the $N(0, D)$ distribution for the random effects b_i.

For the analysis of this data the multivariate model (Bivariate) will be consider as a special case under the generalized linear mixed model. The general multivariate model formulation is given as

Let Y_i be the vector of n repeated measurements for the ith subjects (Weeks):

$$Y_i = \begin{pmatrix} M_{ij} \\ I_{ij} \end{pmatrix}$$

where M_{ij} is the jth outcome for mortality measured for subject i and I_{ij} is the jth outcome for Bluetongue incidence measured for subject i.The general multivariate model assumes that Y_i satisfies a regression model

$$M_{ij} = g(X\beta) + \varepsilon_{Mij}$$
$$I_{ij} = g(X\alpha) + \varepsilon_{Iij}$$

in which g(.) is the inverse link function, and where the error terms have the appropriate the distribution with variance equal to

$$Var(Y_i) = Var(\varepsilon_i) = \Sigma$$

where is $\sum$ is variance covariance matrix.

Since the measurement of the incidence and mortality in the weeks is correlated, it therefore implies a special structure for the covariance structure can be assumed to account for this. Fitting the correct covariance structure to the data will ensure that the standard errors of the models are estimated correctly. The unstructured covariance structure was used for this study and which implies that the variances and the covariance are not same between pairs of measurements.

A Poisson distribution (mean and variance are always equal) is often used in practice to describe count data under homogeneity conditions. However in practice overdispersion (variance-mean ratio larger than 1) is a common phenomenon in count data[1][8]. A distribution often used for overdispersed count is the negative binomial distribution. Negative binomial distribution was used to account for the overdispersion in the data, this was motivated by the fact that the variances of the responses of interest were larger than their respective means (see Section 4.1). The negative binomial distribution has probability mass function

$$f(y;k,u) = \frac{\Gamma(y+k)}{\Gamma(k)\Gamma(y+1)}\left(\frac{k}{\mu+k}\right)^k\left(1-\frac{k}{\mu+k}\right)^y \text{ , y=0,1,2,…,}$$

where k and μ are parameters. This distribution has

$$\mathrm{E}(Y) = \mu, \quad \mathrm{var}(Y) = \mu + \frac{\mu^2}{k}.$$

The index k^{-1} is called a dispersion parameter. As $k^{-1} \to 0$, var(Y) $\to$ μ and the negative binomial distribution converges to the Poisson [1]. Usually k^{-1} is unknown. Estimating it helps summarize the extent of overdispersion.

The log-link (i.e g is the exponential function) function was used in modeling the negative binomial. For simplicity this model allows k to be the same constant for all observations.For the present study, the linearization method was used for fitting the multivariate model. The method of fitting a marginal model using a linearization approach can generally be viewed as an approximation or expansion method. This method consists of linearizing the outcome by using the linear terms of a Taylor series expansion of the linear predictor around the mean, thereby creating a working variate. Iterative reweighted least squares is then applied to obtain the usual components of a multivariate normal model [14].

The procedure GLIMMIX in SAS was used to fit the GLMM.

4 RESULTS

4.1 Exploratory Data Analysis (EDA)

The dataset used in this study consist of bivariate response which are the incidence and mortality. In this section, some EDA techniques were applied to have summary description of the variables used in this study. The descriptive statistics in Table 4 shows that the mean mortality (*BTM*) for species Bovine (25.06) similar to species Ovine (23.56). This may indicate that there is no difference between species mortality. Same scenario was observed for the mean of bluetongue incidence (*INC*) for species Ovine and Bovine. This may indicate that there is no difference between species incidence. It can be observed that the overall mean mortality and incidence was not different from both species mean mortality and incidence. From Table 4 despite that the means were similar for mortality and incidence in both species, their variances were different. The overall mean mortality and incidence are similar to the species mean and incidence mortality. The counts for the incidence were measured in herds and that for mortality were measured as animals. We do have a count data, a Poisson distribution (mean and variance are always equal) is often used in practice to describe count data under homogeneity conditions. It can be observed from Table 4 that the average number of incidence and mortality for species Bovine and for Ovine is less than their sample variance. The sample mean is less than the sample variance implying that there is more variability in the data that a Poisson distribution can explain (overdispersion). This has an implication of the type of model that we will fit to the data sets. A distribution often used for overdispersed count is the negative binomial distribution.

Table 4: Descriptive statistics for Bluetongue Incidence and Mortality

Species	Variable	N	Mean	Variance
Bovine	INC	80	3.71	24.64
	BTM	582	25.06	1633.39
Ovine	INC	79	5.04	46.35
	BTM	583	23.56	1265.42
Overall	INC	159	4.37	35.64
	BTM	1165	24.30	1448.57

Figure 1 shows the histogram of the distribution of mortality and incidence for species Bovine. The number of death for species Bovine ranges from 0 to 237 and the Incidence ranges from 1 to 26 per week for the different provinces. It can be observed from the histogram of incidence that there are some outlying observations.

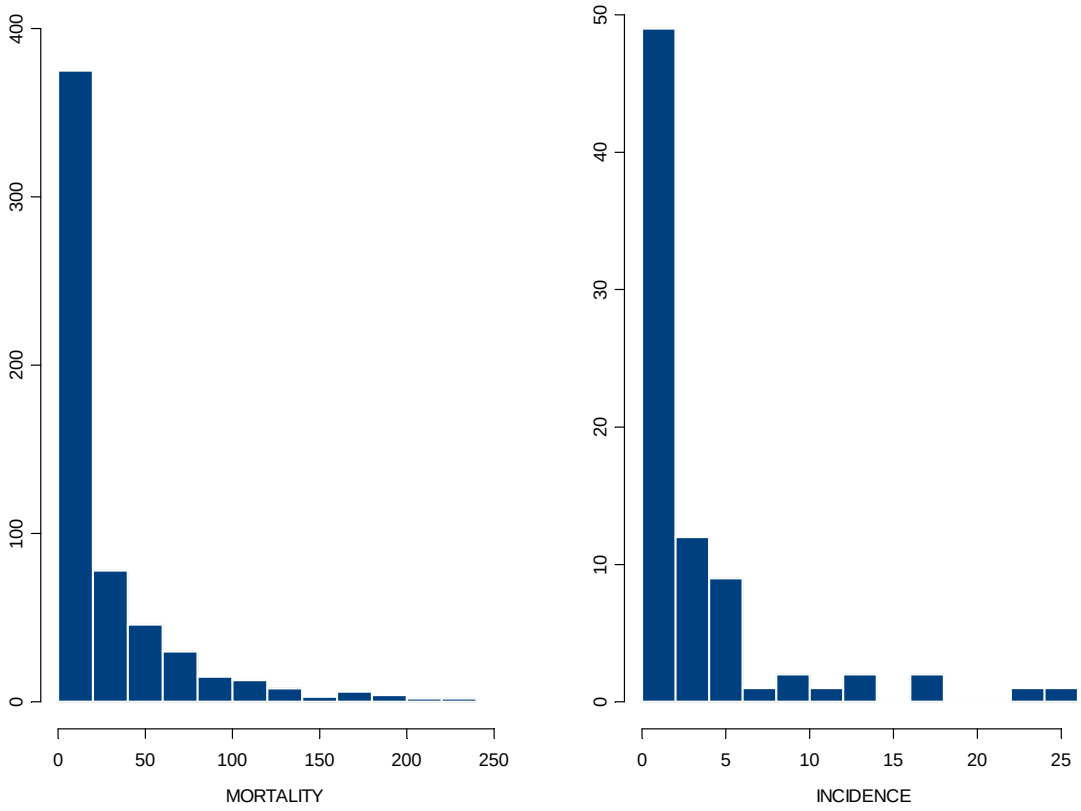

Figure 1: Histogram for Bovine Mortality and Incidence

Figure 2 shows the histogram of the distribution for species Ovine. The mortality ranges from 0 to 417 and the incidence from 1 to 39 per week. It can be observed from the histograms that there are some outlying observations. The frequency distribution of the count is asymmetric as shown in Figure 1 and 2. The frequency decreases as the number of counts increases as seen figure 1 and 2. The same pattern was observed for the overall incidence and mortality as shown in Figure 3 in the appendix.

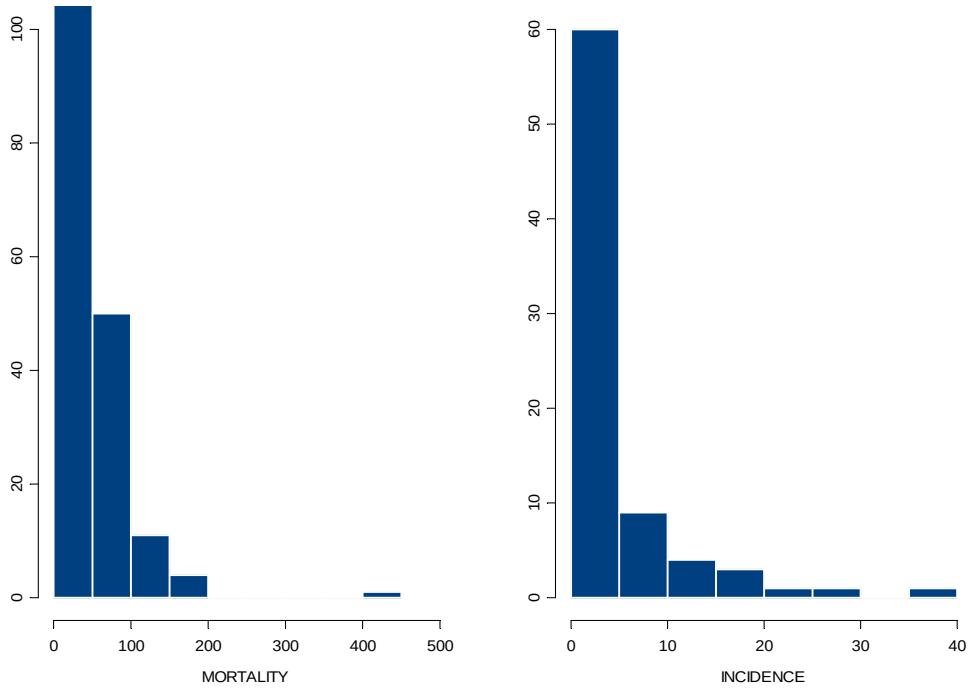

Figure 2: Histogram for Ovine Mortality and Incidence

Table 5 shows the result of the pairwise correlation for mortality and incidence. The pairwise correlation for species Bovine is weak. This may suggest that incidence never had much

Table 5: Pairwise correlation for Mortality and Incidence

		Mortality
Bovine	Incidence	0.39
		0.0004
Ovine	Incidence	0.57
		<.0001
Overall	Incidence	0.50
		<.0001

influence on Bovine mortality. The correlation for Ovine is slightly strong, same as for the overall effect. This may suggest that incidence had a slight influence on ovine mortality as where as for the overall effect.

4.2 Statistical Analysis

In this part the statistical analysis will be presented. A GLMM model using negative binomial as distribution was fitted with the response being the bivariate response value of mortality and bluetongue incidence was model against *Resp*, *Species*, *province* and their interactions.

Table 6: Estimates for Mortality and Bluetongue Incidence

Variables	Province	Species	Resp	Estimate	Standard Error	P-value
Resp			1	3.95	0.11	<.0001
Resp			2	1.02	0.19	<.0001
Resp*species		bovine	1	0.08	0.08	0.2768
Resp*species		ovine	1	0	.	.
Resp*species		bovine	2	-0.13	0.16	0.4192
Resp*species		ovine	2	0	.	.
Resp*Province	ANTWERP		1	-0.52	0.16	0.0015
Resp*Province	BRUXELLES		1	-3.83	0.11	<.0001
Resp*Province	EAST FLANDERS		1	-0.29	0.14	0.0496
Resp*Province	FLEMISH BRABANT		1	-1.13	0.13	<.0001
Resp*Province	HAINAUT		1	-0.93	0.16	<.0001
Resp*Province	LIEGE		1	-0.76	0.15	<.0001
Resp*Province	LIMBOURG		1	-0.68	0.14	<.0001
Resp*Province	LUXEMBOURG		1	-0.56	0.15	0.0005
Resp*Province	NAMUR		1	-0.84	0.23	0.0005
Resp*Province	WALLOON BRABANT		1	-2.24	0.15	<.0001
Resp*Province	WEST FLANDERS		1	0	.	.
Resp*Province	ANTWERP		2	0.42	0.22	0.0698
Resp*Province	EAST FLANDERS		2	1.55	0.25	<.0001
Resp*Province	FLEMISH BRABANT		2	0.01	0.23	0.9684
Resp*Province	HAINAUT		2	-0.84	0.19	<.0001
Resp*Province	LIEGE		2	0.28	0.32	0.3852
Resp*Province	LIMBOURG		2	0.42	0.28	0.1498
Resp*Province	LUXEMBOURG		2	-0.81	0.19	<.0001
Resp*Province	NAMUR		2	-0.57	0.21	0.0081
Resp*Province	WALLOON BRABANT		2	-0.82	0.26	0.0020
Resp*Province	WEST FLANDERS		2	0	.	.

The result of the test of fixed effects of the GLMM model in Table 10 in the appendix shows that there is significant difference between the overall effect of mortality and incidence. The interaction (*Resp*species*) shows that the there is no difference of incidence between species, as well as mortality. This was expected as it was observed from the summary statistics that there

was no difference in mean between the species. Also it can be observed from the Table 10 of the appendix that the interaction (*Resp*Province*) is significant. This indicates that there is a difference in mortality in the provinces for species, same as for incidence.

The results of Table 6 indicates that the negative estimates of the interaction (*Resp*Province*) due to mortality indicates the mortality is lower for all the provinces than the reference province West Flanders. The non-significant estimates for Easter Flanders indicate that there is no difference in mortality with the reference West Flanders. The estimates of the interaction (Resp*Province) due to bluetongue incidence indicates that the incidence was lower for the Provinces Hainaut, Luxembourg, Namur, Walloon Brabant than the reference Province West Flanders. The Bluetongue incidence was higher in the Provinces Antwerp, East Flanders and Limbourg than the reference West Flanders. The Bluetongue incidence in Flemish Brabant and Liege was not different from the reference West Flanders. The interaction (Resp*species) was not significant. This implies there was no difference between the species Bovine and Ovine with to mortality and incidence.

The bivariate nature of the measurements on each week implies that the measurements are correlated and therefore a proper variance covariance structure should be specified to account for this. Fitting the correct covariance structure to the data will ensure that the standard errors of the models are estimated correctly. The unstructured covariance structure was used for this study to account for correlation between incidence and mortality measured for each week. The unstructured covariance structure implies that the variances and the covariance are not same between pairs of measurements. Table 7 shows the covariance parameter from which the correlation can be calculated.

From Table 7 the overall correlation between *Resp1* (mortality) and *Resp2* (Incidence) obtained from the covariance matrix is 0.25 which is a weak correlation. The correlation obtained from the covariance matrix for the different species shows that the correlation for species Ovine is (0.3) and Bovine (0.15). This correlation is the correlation between Resp1 and Resp2 within same week.

Table 7: Covariance parameters estimate for Mortality and Incidence

Parameter	Subject	Group	Estimate	Standard Error
UN(1,1)	Weeks	species bovine	1.6563	0.3793
UN(2,1)	Weeks	species bovine	0.1835	0.1875
UN(2,2)	Weeks	species bovine	0.8008	0.1918
UN(1,1)	Weeks	species ovine	1.3477	0.3187
UN(2,1)	Weeks	species ovine	0.2490	0.1246
UN(2,2)	Weeks	species ovine	0.5242	0.1384
UN(1,1)	Weeks	overall	1.4871	0.2375
UN(2,1)	Weeks	overall	0.2372	0.1013
UN(2,2)	Weeks	overall	0.6195	0.1077

When these correlations are obtained, they can be test if they are significant compare to the banded unstructured covariance (UN (1)) which assumes no correlation. The -2Remllog likelihood ratio test were use to compare the goodness of fit as an approximation. The covariance for group species was compared to the overall covariance to see if the correlation can be better explained by the group species or by the overall effect. From table 8, the results shows that the correlation can be explain by species effect.

Table 8 : Correlation hypothesis testing

Hypothesis	$-2\ln[\lambda_{REML}(\hat{\theta})]$	Chi-square distribution	P-value
$UN_{group\ species}$ vs $UN_{overall}$	30.23	χ^2_3	0.0001
$UN_{group\ species}$ vs $UN(1)_{group\ species}$	16.88	χ^2_1	0.0001
$UN_{overall}$ vs $UN(1)_{overall}$	11.27	χ^2_1	0.008

The result of Table 8 shows that the correlation for the overall and for both species between *Resp1* and *Resp2* are significant when compared to the banded unstructured covariance. Thus one can conclude that there exist a weak correlation between mortality and incidence. This weak correlation may imply that the incidence never had much influence on mortality in 2006.

5 DISCUSSION AND CONCLUSION

Bluetongue can be a costly infection for several reasons. The clinical disease in sheep can be severe, resulting in deaths, weight loss and wool break. Bluetongue is a disease of sheep, but cattle are the principal vertebrate reservoirs of the virus. Once established, it is impossible to actively eradicate bluetongue virus. The virus will circulate, generally subclinically, in cattle and other ruminants, and in midges.

This study focused on establishing if there is a correlation between the incidence 2006 and the mortality in 2006. The secondary objective was to investigate the effects in the different provinces.

To achieve the main objective of this report, the data set was obtain by taking the difference in the 2006 mortality and mean mortality of previous year's (2002 to 2005) was obtained. The assumption is that this difference is the mortality due to Bluetongue. In taking this difference negative values were observe and these values were translated to zero. Before translating it to zero the 2006 mortality values had to fall in the interval of the mean distribution of 2002-2005 mortality and all these values felt in the interval. This assumption may introduce bias in analysising the data. The data was re-arranged such that the time of incidence should match the time of death. It is because when an animal is diagnosed with bluetongue it may take several days before the animal dies. It was found that when a species Bovine was diagnosed it may take 11 weeks before it die while for species Ovine it may take 8 weeks base on the data when using the lead function. However this results differ much from literature and reality, so 1 week consider as time it takes an animal to die after being diagnosed with Bluetongue virus. The data of the Bluetongue incidence was then merge with the difference in mortality obtain for species Bovine and Ovine in bivariate format as a special case of the multivariate model. This data was used to establish the correlation of the Bluetongue and the mortality in 2006. The assumption is that if this correlation exists then Bluetongue did not occur before 2006.

In the Exploratory Data analysis, it was observed that the mean of the incidence and mortality for both species did not differ much same as with the overall mortality and incidence. The mean of

the response variables in the data sets were smaller than their variance of the variable which implies there is overdispersion in the data and this has an important implication for the type of model to fit for the data. Since the response of interest, mortality and Bluetongue incidence are count variables and can be model by Poisson distribution. However, base on the assumption of equal mean and variance of Poisson distribution, there is overdispersion in data since the mean is smaller than the variance. This is implies that there is extra variability in the data that cannot be captured with a Poisson model and the negative binomial was used as a natural solution for overdispersion.

In analyzing these data sets statistically, the generalized linear mixed model was used and with negative binomial as distribution. A strong assumption used in this analysis is that we have a constant population. Since are bivariate response is a correlated measurement for the subject weeks. A good covariance structure is needed to model this correlation and the unstructured covariance structure was used which assumes different parameter for the different measurements. This correlation was taken into account by modeling the residual correlation under the GLIMMIX procedure in SAS. The result of the statistical analysis shows that there is significant difference between mortality and bluetongue incidence in 2006 and in the provinces. It was observed that there is no difference of mortality between species, as well as incidence in species. This was expected as it was observed from the summary statistics that there was no difference in mean between the species. The overall correlation without considering species effect between mortality and incidence is 0.25 which is a weak correlation. When species effect was considered, the correlation for species Ovine was 0.30 and Bovine was 0.15.To test if this correlation was significant the likelihood ratio test was used to compared the full unstructured covariance structure and the banded covariance structure (UN (1)). From this result of the correlation one can conclude that there is a correlation between mortality and incidence within the same week However caution has to be taking when interpreting this correlation since the test used in establishing it is an approximation.

In order to carry out this analysis are there some assumptions made which can be considered as bias. The assumption made to translate negative values to zero when the difference in mortality was obtain between 2006 and previous mortality implied that in these weeks they were no

mortality observed which is not true. Also the mean mortality of 2002-2005 introduce fractions and these were rounded up which maybe biasing the result. The approximation made in establishing the time of death when an animal is infected by Bluetongue virus maybe bias since it is data driven. Also the 1 week taken as the time to death for both species may be bias as it is known from literature that Bovine are the reservoirs for Bluetongue virus. It would have been proper if animals were followed up in a cohort study. Also another assumption made was on the mortality data for Ovine. This data was for Sheep and Goat but it was difficult to separate the mortality between Sheep and Goat to know which observation were for Sheep and which were for Goat , while the incidence data was for Ovine (sheep). These maybe biasing the result since the right observations were not matched between mortality and incidence. Maybe this has an effect on the non significance difference in mortality and incidence between the species Bovine and Ovine.

In this study the correlation between the mortality and incidence in 2006 was investigated and there was correlation between the mortality and incidence. Thus the results of the study seem to show that Bluetongue had no influence on ruminant mortality during the year 2006. There is no difference of incidence between species, as well as mortality. There is difference in mortality and incidence in the various provinces. However caution has to be taking when interpreting this correlation since the test used in establishing it is an approximation.

6 RECOMMENDATION

In this study although it has been establish that there is a correlation between mortality and incidence in the year 2006. Clearly great caution has to be taken with the result of this study for reference purpose. Since the test used in establishing it is an approximation. However it is pertinent to make some recommendation.

Firstly, when carrying an analysis, appropriate technique(s) should be used in order to be able to come up with valid results. However this data had some limitations. Deaths should be recorded from the same herds all year round and measure in each herd, the incidence and mortality. Better data validation and consistency should be ensured. Also, for future data collection and for better comparison it will be proper if these observations came from the same herds so as to reduce the variation between the herds. .

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APPENDIX

Table 9: The different lead time for Bovine and Ovine Mortality

BOVINE		
Weeks	P-value	AIC
1	0.0028	674
11	0.0035	601.14
OVINE		
Weeks	P-value	AIC
1	0.042	697
8	0.0032	668.69

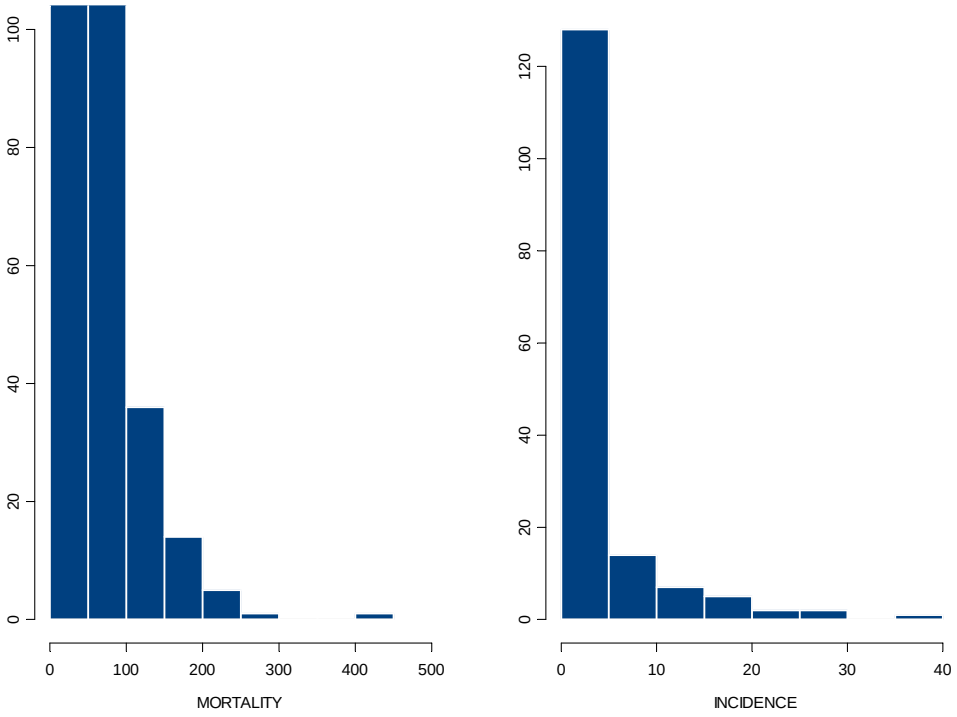

Figure 3: Overall effect of Mortality and Incidence

Table 10: Mortality and Incidence fixed effects test

Parameters	P-value
Resp	<.0001
Resp*species	0.3683
Resp*Province	<.0001