Probability of infection with Johne's disease in dairy cows coming from infected dams versus uninfected dams

Source: Clarke (1997)

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Preface

Research comes from the Middle French word: recherche, from the verb rechercher: to search for. It is a way to find answers in multiple areas of expertise by gathering data, information and facts to gather knowledge about a subject.

This report is the result of research committed by M. de Goeij. This research was done at the University Utrecht, department of Farm Animal Health in the context of a research internship, obligatory in the function-based phase of the study of veterinary health.

Data was gathered by students, veterinarians and researchers working at the department of Farm Animal Health. Collected information was analyzed using multiple approaches to conduct research on the probability of infection in offspring of infected vs. uninfected dams.

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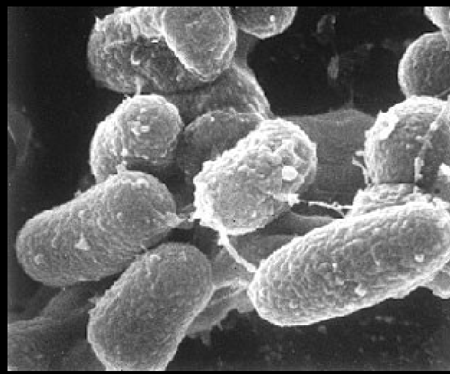
Summary

The most profound route of transmission of Paratuberculosis is oral uptake of the organism through milk or feces. However vertical transmission is suspected. This study aimed to compare the probability of infection in calves coming from infected dams versus uninfected dams. The data set contained milk and serum samples collected during routine milk research and collected by researchers. This was combined with pedigree data of 13097 cows to find mother-daughter combinations. Giving information to construct a 2x2 table which was used to perform a Fisher's exact test to calculate a P-value and a confidence interval (CI): 0.079, 95%-CI: (-0.023; 0.157). Also the odds ratio was calculated with a CI: 2.309, 95%-CI: (0.801; 5.868). Neither being significant. Next a model was used to measure the influence of the infection status of the dam using repeated measures of the milk ELISA's and MPRdata of 1388 cows. Giving a least square means of 2.6511 for a negative dam, 2.7043 for a positive dam and 2.6307 for missing dam infection status. However these results were not significant and due to intertwinement between dam infection status and herd and a necessary log-transformation of the s/p ratio these results do not represent an accurate assessment of the parameters.

Introduction

Causative agent

Paratuberculoid organisms were first found by A. Johne and L. Frottingham in 1895. Soon afterwards, in 1910, the organism was cultured and classified as a mycobacterium by Twort and Ingram. Since then the organism was named several times differently, most recently it became: *Mycobacterium avium* subspecies *paratuberculosis* (MAP). MAP causes paratuberculosis in domestic and wild ruminants and rabbits, also known as Johne's disease. Despite the fact paratuberculosis was first described in cows already over a century ago, until today no therapy exists and many mysteries remain (Clarke, 1997).



Source: http://microbewiki.kenyon.edu

Figure 1: Scanning electron micrograph of *M. paratuberculosis.*

MAP is a small acid-fast bacillus, depicted in Figure 1. Its cell wall contains many lipids, making it very resistant to physical influences such as pH and temperature changes and low availability of water or nutrients. It can survive in the environment in soil or feces probably for years but up to eight months has been reported (Koets, 2000).

Route of infection

In cows oral ingestion of the organism through milk, food or licking the environment and in particular close contact of susceptible animals with contaminated feces is now believed to be the primary important route of transmission (Hoek, 2009). Recently Eisenberg et al., 2010 showed that MAP can survive and spread in dust. Vertical transmission has been described as well (Whittington, 2009; Sweeney, 1992).

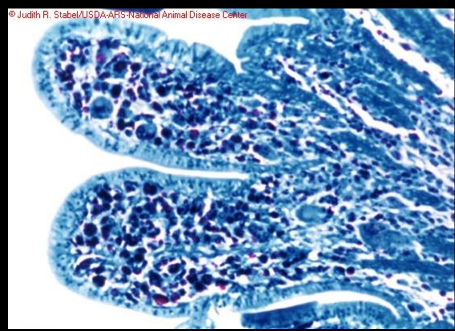
Source: http://microbewiki.kenyon.edu

Figure 2: Cow with clinical signs of Paratuberculosis

In an infected environment, newborn calves are likely to get in contact with MAP. Though many get infected, most calves are able to expel the bacteria by a protective immune response. Others become chronically infected and enter a subclinical phase. This subclinical phase may last forever, but can be as short as two years. Of these subclinically infected animals, 10-15% are likely to become clinically ill (Over, 2011). Factors influencing the length of the subclinical phase may be the infection dose and age at the time of infection. Most animals becoming clinically infected do so at the age of four to five years and clinical symptoms are often detected after experiencing stress factors such as calving or high production (Koets, 2000).

Infected cattle can be categorized in four stages. Stage I usually represents infected animals up to two years of age. They have no signs of illness and infection cannot be reliably detected. In stage II, no apparent symptoms of infection are present but animals may have a decreased reproductive performance. They intermittently shed bacteria. In stage III and IV the animals become clinically and advanced clinically ill (Figure 2). They shed large numbers of bacteria (Barrington, 2003).

After oral infection MAP survives and replicates in the macrophages in the intestinal wall (Stabel, 1998). Within a month lesions can be found in several lymph nodes including hepatic, mesenteric, suprapharyngeal and ileoceacal lymph nodes. Also tonsils, ileum and caecal valve may already be affected. As the infection progresses the infection spreads from the lymphoid tissue of the intestinal Peyer's patches to other Peyer's patches, in particular the ileal Peyer's patch. Since MAP resides intra-cellularly, cell-mediated immunity is most important, the ileal Peyer's patch seems to have less T-cell rich areas which may be the cause of the lesions being more apparent here (Clarke, 1997; Kreeger, 1991). At 6-15 months of age the ileal Peyer's patches are disappeared after regressing and jejunual patches remain (Koets, 2000). Lesions include an extensive granulomatous inflammation in distal part of the ileum (Figure 3), causing protein losing enteropathy and malabsorption, explaining the symptoms: weight loss, decreased milk production and diarrhea. After intestinal lesions have developed the animals start shedding organisms in the feces though this is still minimal while in the subclinical phase. When an animal becomes clinical, shedding might exceed 10^8 organisms/g feces (Stabel, 1998).



Source: http://www.cabi.org

Figure 3: Cross-sectional view of ileum of MAP infected sheep, acid-fast stained

Diagnostic tests

The diagnosis of paratuberculosis is based on two approaches: detection of immune response of the host or detection of the etiologic agent. Most commonly applied methods are ELISA in milk or serum, fecal culture, and PCR. All have benefits and disadvantages. Diagnostics can be used to confirm the diagnosis in suspected animals, for herd screening and to detect subclinically infected animals. Definitive diagnosis is now based on fecal culture or histology post mortem.

ELISA is used to detect MAP specific antibodies in the host. Milk ELISA is very suitable for herd screening since milk samples are already collected routinely on many farms. These tests have high specificity (94.6% has been reported (Lombard, 2006)) but very low sensitivity (60.9% has been reported (Lombard, 2006)). Milk ELISA is a less labor intensive method than serum ELISA, and milk ELISA seems just as sensitive as serum ELISA (Lombard, 2006). Though the manufacturer claims serum ELISA has a slightly higher sensitivity (IDEXX, Hoofddorp, The Netherlands, competitive information sheet, MAP ab test). Fecal culture methods are getting more sensitive with improving purification techniques and different growth media (Eamens, 2000). Also incubating time is getting shorter though still a minimum of six weeks is required ELISA on the other hand is relatively fast. PCR can be done on several tissues as well as on milk, feces and other samples. Though it has a high specificity and speed also this test is limited due to low sensitivity. Moreover, the test is too expensive and complicated to be used for routine diagnostics. PCR has also been investigated in sheep but seems to be less sensitive than histology post mortem (Gwozdz, 2000). All tests are influenced by the intermittent shedding and daily variation and all tests are more sensitive after an animal gets clinical (Barrington, 2003). Furthermore the most sensitive diagnostic tests are post-mortem examinations which are less valuable to farmers concerned about their current herd prevalence (Nielsen, 2007).

Due to the long incubation period of paratuberculosis, the low sensitivity of the diagnostic means and difficulties in recognizing and reporting the disease it has been hard to accurately determine the prevalence (Stabel, 1998). In 2000 research in the Netherlands found a herd prevalence of 55%, meaning 55% of the herds tested had one or more positive cows, tested by serum ELISA. The true prevalence in the Netherlands on cow level was estimated at 2.7 – 6.9% and the prevalence on herd level 31 – 71%. An accurate estimation on true prevalence is hard because of the low test sensitivity (Muskens, 2000). USA dairy studies found a herd prevalence in 2002 and 2007 respectively of 20-40% and 68%. Suggesting that prevalence of infection may increase (Hoek, 2009). These numbers show paratuberculosis is widely spread in ruminants and even though the mortality rates are not that high, gives reasonably high economic losses in dairy cattle due to a fall in milk yield, weight loss without loss of appetite, diarrhea and the costs of laboratory testing and control measures (Clarke, 1997).

MAP also has been isolated from ileal lesions in human patients with Crohne's disease. Furthermore Crohne's disease in humans has several similarities with Johne's disease in ruminants. For example both diseases cause chronic enteritis by granulomatous inflammation of the intestinal wall (McFadden et al., 1987; Stabel, 1998). For these reasons MAP is by some considered a potential zoonosis making the reduction of human exposure via consumables a public health issue (McFadden et al., 1987).

Control of paratuberculosis

Treatment of paratuberculosis is expensive and mostly ineffective. Though standard anti-tuberculosis drugs can give clinical improvement in the individual animal, shedding continues. In goats a 60 day combination treatment was successful, improving the health of the animals and clearing the organism shedding. However because of the extended period of therapy and the costs, treatment is not considered a viable alternative for euthanizing (Stabel, 1998).

Another option for reducing paratuberculosis would be vaccination. However, in the Netherlands the government demands no interference with the diagnosis of bovine tuberculosis and vaccination is restricted. In the past herds with severe clinical problems were allowed to vaccinate (Muskens, 2002). Till now killed vaccine does not prevent transmission and does not decrease herd prevalence (Kalis, 2001). Therefore no vaccines are currently registered against paratuberculosis for cattle in the EU, and vaccination is not allowed (Santema, 2011).

Since no therapy or vaccine is available, disease control is mainly based on prevention by hygiene and test and cull. And several programs are developed to aid farmers in reducing paratuberculosis in their herd. Current strategies for control in the Netherlands are based on preventing calves to have contact with contaminated feces, identification of subclinical cases by individual milk and serum sampling, and hygienic calf rearing (Dutch Animal Health Service, Deventer, the Netherlands). Since susceptibility is believed to be highest in calves and is nihil at one year of age, this should prevent development of new paratuberculosis infected cows, causing the disease to diminish. In the Dutch program only the orofecal route is taken into account. Also abroad these programs exist, in Australia however, farmers seem to know about the importance of paratuberculosis but the majority does not comply with most of the recommendations with regard to calf rearing (Wraight, 2000). Therefore the failing result of eradication programs might be due to lack of accurate application of measures. Another reason might be the combination of a long incubation period with the very resistant nature of the bacillus that makes it hard to evaluate the effect of a program as it may take years for any effect to become apparent.

In conclusion, treatment and classical control strategies as vaccination, hygiene and test and cull are not able to eradicate disease. Therefore, new approaches to contribute to control of disease will be explored and further research to known routes of transmission will be deepened.

The aim of this study is to contribute to current knowledge about vertical transmission. In this study, the probability of infection with Johne's disease in dairy cows coming from infected dams versus the probability of infection in cows from uninfected dams will be determined. This knowledge will contribute to the current understanding about whether or not early post-partum infection and intra-uterine infection with Johne's disease can take place.

Materials and Methods

Analysis was done in two parts. First, pedigree data and serum and milk ELISA results were combined and sorted in a 2x2 table for application of a Chi^2-test to find out if the probability of infection with Johne's disease differs in cows coming from infected dams versus cows coming from uninfected dams. Second, a mixed model was used to estimate the effect of the infection status of the dam on the level of MAP specific antibodies in the milk while correcting for environmental/animal factors influencing the level of antibodies.

2x2 Table

Samples

Milk samples were collected from lactating cows during the routine milk production scheme. From October 2009 until October 2011, milk samples were sent to the Faculty of Veterinary Medicine to be tested for antibodies specific for Johne's disease by a commercially available ELISA (ELISA Paratuberculosis Antibody screening, IDEXX, Hoofddorp, the Netherlands) according to the instructions of the manufacturer. Additional serum samples were collected from cows that tested positive in the milk ELISA and serum ELISA was performed to confirm infection status. Outcome of the ELISA was a sample to positive ratio (s/p value). All serum ELISA's were done in double, the mean of these two values was calculated and compared to the cut-off value for being positive, to be found in Table 1. Questionable outcomes were considered negative, making our cut-off value for being positive in milk 30% and for being positive in serum 55%.

	Milk	Serum
Negative	<20%	<45%
Questionable	20-30%	45-55%
Positive	>30%	>55%

Table 1: s/p values and the corresponding outcomes

If the serum sample tested positively, collection of serum samples was continued during life of the cow. If the serum sample tested negatively three times in a row, serum collection stopped, cows were considered uninfected and went back to only milk ELISA surveillance. In total, approximately 1378 cows originating from eight commercial dairy farms high prevalent for Johne's disease in the Netherlands were included in this study.

Data edits

Pedigree was provided by the Dutch Cattle Improvement Organization (CRV, Arnhem, the Netherlands). The pedigree contained data on 13097 animals providing information on the dam of the animal as well as its sire, date of birth, breed and gender. Pedigree information was used to connect dams and daughters which were both in the test data.

First, test data was used to determine whether an animal was infected or not. In total, 1378 animals were repeatedly tested in milk for antibodies. If tested positively, the animal was tested in serum. Then an binary trait was assigned to an animal: if an animal tested positively once in serum ELISA, it was characterized as being positive (infected); 1. If an animal tested negatively in serum multiple times, it was characterized as negative (uninfected); 0. Based on these restrictions, data used for analysis contained 129 positive animals. Infection status was matched to the dams in the pedigree and dam with infection status was added to the animal record in the test data. Only animals of which the dam infection status was available were taken into account. Giving a total of 486 animals which were either a dam, a daughter or both in this project.

Statistical analysis

The aim of this study was to investigate if the probability of infection with Johne's disease in dairy cows coming from infected dams differs from the probability of infection in cows from uninfected dams. To be able to answer this question, a hypothesis was postulated:

$$H_0 = p_1 = p_2$$
$$H_1 = p_1 \neq p_2$$

p_1 being the chance an animal is infected, coming from an infected dam and p_2 being the chance an animal is infected coming from an uninfected dam. To obtain these probabilities observed data had to be organized in following 2×2 table (Table 2):

OBSERVED	DAMS			
ANIMALS		Infected	Uninfected	**Total**
	Infected	a	b	**a + b**
	Uninfected	c	d	**c + d**
	Total	**a + c**	**b + d**	**a + b + c + d**

Table 2: 2 x 2 table. a = number of infected animals coming from an infected dam, b = number of infected animals coming from an uninfected dam, c = number of uninfected animals coming from an infected dam, d = number of uninfected animals coming from an uninfected dam.

Probability to become infected in the two different daughter groups could be calculated using following formulas:

$$p_1 = \frac{a}{(a+c)} \text{ and}$$

$$p_2 = \frac{b}{(b+d)}.$$

This was all done for all animals in the dataset as well as for every farm separately.

Expected data were calculated to obtain the P-value using the following formulas (Table 3). This data will be used to apply a Chi^2-test or Fisher's exact test depending on the values for the expected data. If one of the values of the expected data is under five, Chi^2-test cannot be used and Fisher's exact should be used instead.

EXPECTED	DAMS		
ANIMALS		Uninfected	Infected
	Infected	$\frac{(a+c)(a+b)}{n}$	$\frac{(b+d)(a+b)}{n}$
	Uninfected	$\frac{(a+c)(c+d)}{n}$	$\frac{(b+d)(c+d)}{n}$

Table 3: calculations of the expected values of Table 2.

Next the 95%-confidence interval (CI) was calculated using following formula:

$$CI = (p_1 - p_2) \pm 1{,}96\sqrt{\frac{p_1(1-p_1)}{n_1} + \frac{p_2(1-p_2)}{n_2}},$$

where n_1 and n_2 represent the number of animals in the group of daughters with an infected dam (n_1) versus the number of daughters with an uninfected dam (n_2).

Then, odds ratio (OR) and its corresponding confidence interval will be calculated using the following formulas or by using R depending on whether an exact test is necessary.

$$OR = \frac{\frac{a}{c}}{\frac{b}{d}} = \frac{a \times d}{b \times c}, \qquad CI_{OR} = e^{\ln OR \pm 1{,}96 \times SD}$$

Here the OR represents the odds of exposure in the infected daughters divided by de odds of exposure in the uninfected group.

Finally the attributable risk (R_{att}), the difference in rate of infected calves between the infected population and the uninfected population, was calculated:

$$R_{att} = \frac{\left(\frac{a+c}{a+b+c+d}\right) - \left(\frac{b}{b+d}\right)}{\left(\frac{a+c}{a+b+c+d}\right)} \times 100\%.$$

The analysis was done using R (2.0.0.7). The R-script is attached in Appendix I.

Model

Samples

Milk ELISA results were used and combined with milk production records (MPR data) obtained on the test day provided by the Dutch Cattle Improvement Organization (CRV, Arnhem, the Netherlands) to be able to correct for environmental/animal factors affecting S/P ratio. Data contained 19592 ELISA test results in milk of 1391 cows. Of these, 17605 records could be matched to the MPR data and 145 records could not be used because of missing data in the MPR file. 17460 records of 1388 cows were used for analysis. Dam infection status was available for 484 cows in this data of which 54 had a positively tested dam, 430 had a negatively tested dam and for 904 cows dam infection status was missing.

Data edits

Histograms were made of model variables to ensure at least five measurements in each class. Classes were determined in the framework of biological knowledge (Appendix II).

Statistical analysis

The following model was applied to determine the effect of dam infection status on S/P ratio:

$$\ln(Y_{ijklmnopq} + 10) = A_i + P_j + UBN_k + YOB_l + DIM_m + MY_n + PP_o + DAM_p + animal_q + e_{ijklmnopq}$$

, where $Y_{ijklmnopq}$ is the s/p ratio resulting from the ELISA in milk. Plots of observed versus fitted values indicated that a natural log transformation was needed to satisfy the assumptions with respect to the error terms (Appendix III). All S/P ratios were raised by 10 to avoid negative numbers and maintain all records for the analysis. Besides, original ranking of S/P ratios included in the data has been maintained which is important because variation in S/P ratio reflects a biological difference. A_i is *i*th age of the cow on test day (*i*=≤2, 3, 4, 5, and ≥6); P_j is the *j*th parity of the cow on test day (*j*=1, 2, 3, 4, 5, and ≥6); UBN_k is the effect of the *k*th herd (*k*=A, B, C, D, E, F, G, H, I). One herd moved during this research resulting in nine unique herd numbers. Since the management was different and exposure to the causative agent may be different on the two locations, this herd was included as two different herds. YOB_l is the effect of the *l*th year a cow was born (*l*=1997, 1998, 1999, 2000, 2001, 2002, 2003, 2004, 2005, 2006, 2007, 2008, 2009); DIM_m is the effect of the *m*th lactation stage in classes (*m*=0-10, 10-20, 20-30, 30-40, 40-50, 50-60, 60-90,90-120, 120-150, 150-180, 180-210, 210-240, 240-270, 270-300, 300-350, 350-400, ≥450); MY_n is the effect of *n*th milk yield, PP_o is the effect of the *o*th protein percentage in milk on the test day, DAM_p is the effect of the *p*th infection status of the dam (*p*=0, 1, 2); $animal_q$ is the random effect of the *q*th animal; and $e_{ijklmnopq}$ is the random residual component. The following distributional assumptions were made with respect to the random effects:

$$animal \approx \left(0, I\sigma^2_{animal}\right), \text{ and}$$

$$e \approx N\left(0, I\sigma^2_e\right),$$

where *animal* is the random effect of the animal and e is the random residual effect. σ^2_{animal} represents the animal variance, *I* is the identity matrix and σ^2_e represents the residual variance.

The model was fitted in SAS 9.2 (SAS Institute Inc., 2010), using the Akaike's Information Criterion (AIC) to evaluate the best model. The F-values and P-values of the type III tests of fixed effects of various models can be found in Appendix IV. Results were also used to find correlations between factors affecting S/P ratio. Finally, a subset was made containing only data for the animals with a known dam infection status. The best model according to the AIC was also be applied to this dataset.

Results

2x2 Table

Overall results

Table 4 shows the distribution of daughter-dam combinations with available infection status in a 2×2 table.

OBSERVED	DAMS			
ANIMALS		Infected	Uninfected	**Total**
	Infected	7	25	**32**
	Uninfected	49	405	**454**
	Total	**56**	**430**	**486**

Table 4: observed frequencies in total population

With this data the p_1 and p_2 could be calculated:

$$p_1 = \frac{7}{(7+49)} = 0.125 \quad p_2 = \frac{25}{(25+405)} = 0.058$$

Showing the probability of being infected coming from an infected dam is 12.5% and the probability of being infected coming from an uninfected dam is 5.8%.

Using these values the expected values were calculated resulting in the values as pictured in Table 5:

EXPECTED	DAMS		
ANIMALS		Infected	Uninfected
	Infected	$\frac{(7+49)(25+57)}{486} = 3.687$	$\frac{(25+405)(25+7)}{486} = 28.313$
	Uninfected	$\frac{(7+49)(405+49)}{486} = 52.313$	$\frac{(25+405)(405+49)}{486} = 401.687$

Table 5: expected frequencies in total population

Since one of these values is below 5, the choice was made to use Fisher's exact test instead of the Chi^2-test to obtain a P-value using R. As a result we found a P-value of 0.079. With a significance level of 5% we could not reject the H_0-hypothesis.

Now the 95%-confidence interval (CI) was calculated using following formulas:

$$CI = (0.125 - 0.058) - 1.96\sqrt{\frac{0.125(1-0.125)}{56} + \frac{0.058(1-0.058)}{430}} = -0.023$$

$$CI = (0.125 - 0.058) + 1.96\sqrt{\frac{0.125(1-0.125)}{56} + \frac{0.058(1-0.058)}{430}} = 0.157$$

95%-CI = (-0.023; 0.157). Since p1-p2 would be 0 if H_0 were true, and 0 lies within the confidence interval we cannot reject the H_0-hypothesis according to this data. Meaning no significant difference was found between the probabilities in the two daughter groups.

Since the numbers were too small, an exact test had to be used for the OR and its CI. Giving an OR of 2.309 with 95%-CI = (0.801; 5.868). Since 1 is in this interval, the H_0-hypothesis cannot be rejected, based on the OR.

Last the attributable risk was calculated:

$$R_{att} = \frac{\left(\frac{26}{486}\right) - \left(\frac{20}{431}\right)}{\left(\frac{26}{486}\right)} \times 100\% = 13.3\%$$

An attributable risk of 13.3% means 13.3 percent of infected animals were infected as a result of having an infected dam. This however does not give any information on whether this was caused by a possible genetic component or by maternal transmission.

Results per farm

OBSERVED	DAMS	Uneken		
ANIMALS		Infected	Uninfected	Total
	Infected	1	0	1
	Uninfected	12	38	50
	Total	13	38	51

$P_1 = 0.077$, $p_2=0$

OBSERVED	DAMS	De Jong		
ANIMALS		Infected	Uninfected	Total
	Infected	0	1	1
	Uninfected	2	68	70
	Total	2	69	71

$P_1=0$, $p_2=0.014$

OBSERVED	DAMS	Eggenkamp		
ANIMALS		Infected	Uninfected	Total
	Infected	0	2	2
	Uninfected	9	80	89
	Total	9	82	91

$P_1=0$, $p_2=0.024$

OBSERVED	DAMS	Vd Veen		
ANIMALS		Infected	Uninfected	Total
	Infected	2	2	4
	Uninfected	4	40	44
	Total	6	42	48

$P_1=0.33$, $p_2=0.048$

OBSERVED	DAMS	Dijkstra		
ANIMALS		Infected	Uninfected	Total
	Infected	3	3	6
	Uninfected	9	25	34
	Total	12	28	40

P_1=0.25, p_2=0.107

OBSERVED	DAMS	Krikke		
ANIMALS		Infected	Uninfected	Total
	Infected	0	5	5
	Uninfected	7	56	63
	Total	7	61	68

P_1=0, p_2=0.082

OBSERVED	DAMS	Neimeijer		
ANIMALS		Infected	Uninfected	Total
	Infected	0	1	1
	Uninfected	1	66	67
	Total	1	67	68

P_1=0, p_2=0.015

OBSERVED	DAMS	Menken		
ANIMALS		Infected	Uninfected	Total
	Infected	0	6	6
	Uninfected	5	38	43
	Total	5	44	49

P_1=0 ,p_2=0.136

Model

Factors to be included in the model were determined by backwards elimination. Model 1, 2, 4 and 10 fitted best according to the AIC, all giving an AIC of less than 20200 (Appendix IV).

In model 1 all variables were entered to get an idea of the importance of each variable for the level of MAP specific antibodies in milk (S/P ratio). Age does not contribute much to the variation in S/P ratio. A model without age (model 2) results in a lower AIC. The F-value for parity rises from 18.52 to 35.09 indicating confounding between age and parity. Most of the variation resulting from age also can be traced back to parity and vice versa. This can be explained by the fact that if the age of the animal increases the parity of the animal increases as well. This ascent is nearly linear (Figure 4).

In model 2, YOB and PP are the two least significant variables. Excluding YOB (model 4) results in an even lower AIC. However, YOB showed significant contribution to S/P ratio in model 1 therefore we decided to include YOB in the final model. Besides, we expect differences in exposure to the causative agent in the different YOB. Therefore, the choice to included YOB in the final model has also a biological background. As we would expect confounding between age and YOB, this is not supported by comparing model 2 and 4.

Model 10 shows that the AIC decreases even more if both YOB and age are not included, however for the reasons mentioned above a model including YOB was preferred.

Finally, the model used for analysis included the following variables: DAM, Parity, YOB, UBN, DIM, MY, PP (model 2).

F-values for MY and DIM in model 12 and 13 compared with model 2 indicate confounding between MY and DIM. Correlation between MY and DIM is depicted in Figure 5. The milk production of a cow is very dependent on the stage of lactation, giving a rise in the first period but a gradual decrease of milk yield with progressing lactation stage.

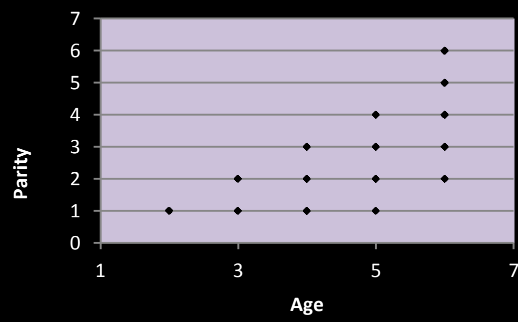


Figure 4: Age vs. Parity

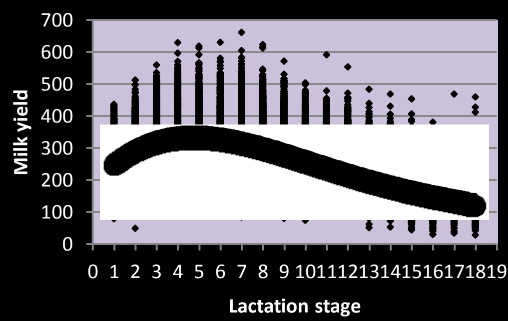


Figure 5: Lactationstage vs. Milk yield

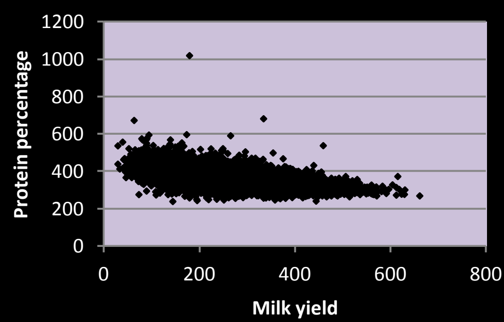


Figure 6: Milk yield vs. Protein percentage

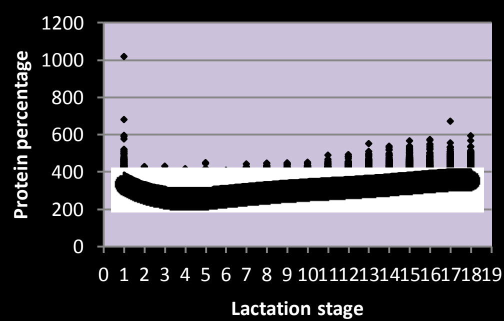


Figure 7: Lactation stage vs. Protein percentage

F-values for MY and PP in model 13 and 14 compared with model 2 indicate confounding between MY and PP. Correlation between MY and PP is depicted in Figure 6. F-values for DIM and PP in model 6 and 8 compared with model 2 indicate confounding between DIM and PP. Correlation between DIM and PP is depicted in Figure 7. DIM and MY have an influence on the PP in the milk. If the MY increases the PP decreases (Figure 6), and with progressing lactation the PP first decreases after which a gradual increase starts (Figure 7). The peak in PP is probably due to colostrum containing a lot of fat and antibodies, as well as other proteins.

UBN is confounded with MY as well as with infection status of the dam, which can be explained by different types of management. It can be expected that cows on a farm with good management produce more milk than cows on a farm with bad management. Infection status of the dam can also be explained by management. Farmers with good management probably cull infected dams more strictly. Also age and parity are confounded with the infection status of the dam. This may be due to the study design. In this study, all cows in lactation on eight farms were monitored for two years, and cows were not monitored from birth till death. Age difference of an infected cow with her dam of known status had to be at least two years. Additionally, only producing cows were included which makes the minimal age of the dam 3.5 years. Most infected cows are detected at an age of three to four years and most farmers cull infected cows. Therefore, if a dam was older and the status of the dam was known, the dam usually was not infected since it would have been culled. This influenced our data reasonably (Figure 8).

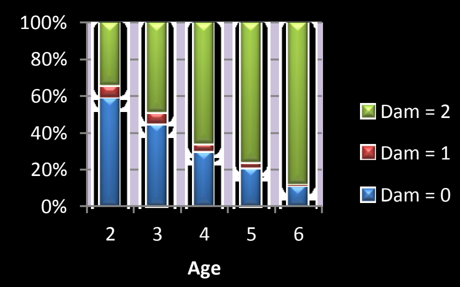


Figure 8: Frequencies of age and dam infection status combinations. 2 = data for dam infection status is missing, 1 = dam tested positively, 0 = dam tested negatively

The least square means for dam infection status are 2.6511 for a negative dam, 2.7043 for a positive dam and 2.6307 when the dam infection status was missing.

However because of confounding between UBN and dam infection status and log-transformation of the response variable, the estimates and least square means for model variables as depicted in Appendix V are not accurate assessments of these parameters.

Additional analysis was done with a subset of data containing only animals with a known dam infection status (6086 observations from 483 animals). These results can be found in Appendix VI and as model 15 in Appendix IV. Leaving out animals with a missing dam infection status gives a significant rise in the F-value as well as a great decrease in the P-value for dam infection status. The variance explained by this model drops from 23 to 16%. This however is not very surprising since the estimates are based on a subset containing about one third of the total data, making the estimates for other variables in the model less accurate. The least square means in this model for dam infection status are 2.8538 if the dam was negative and 2.9331 if the dam was positive. Comparing this to the least square means for the total data set. It can be seen also here the S/P-ratio seems lower in animals with a positive dam.

Discussion

These results do not give any information on whether or not infection in utero exists. Since data collection started when a cow started lactation, usually at a minimum age of two years. However, infection takes place early in life and calves can get infected through the environment as well as through colostrum since this is a route that can hardly be prevented. Also it cannot be controlled whether the farmers applied the measures to prevent calves getting infected accurately (Wraight, 2000).

Koets et al. (2000) showed a protective effect on calves coming from an infected dam. Yet it is currently believed infected dams have a negative effect on their calves and it is advised to cull calves coming from an infected dam. Results of this study show no difference in probability of infection in cows coming from infected dams versus uninfected dams. Nevertheless, Table 4 shows that the group of infected animals coming from infected dams is very small, rising suspicion that a protective effect may yet exist, yet looking at the probabilities it seems the probability of getting infected coming from an infected dam seems twice as big as the probability coming from an uninfected dam. These groups however were too small to make accurate assumptions based on this data. Also the least square means suggest a positive effect of the dam being infected on the calves, however also this data is not significant. Maybe if the research were prolonged, more infected animals would have been detected since about 20% of the measures were made on animals younger than three years old, an age at which MAP can be missed very easily. So many of the subjects may still have been in their incubation period not excreting any antibodies against MAP.

Another reason for infected animals not being detected is the low sensitivity and high specificity of the available tests. The animals testing positively in milk and serum can be assumed to be MAP positive, animals testing negatively in milk might however be false negatives. If an animal did not test positive in milk it was never tested in serum and it was classified as negative in this research. Making it reasonable some positive animals were missed in this research.

When plotting the residuals it can be seen that, even though a log-transformation has been applied, there is still some positive skewness, making the estimates not completely accurate.

No significant effect of the infection status of the dam on the s/p-ratio in the animals can be found when applying the mixed model. Probably for the same reason as why no significance could be fond be before: because of the little amount of infected animals and dams in this study. To get more a better idea about the difference, more infected animals would be needed and longitudinal data over a longer time span.

If results of the model including all data is compared to results of the model including just animals with a known dam infection status it becomes clear the lack of significance is also a result of too little information on the status of the dam. Giving rise to the assumption that to find accurate numbers on the risk of infection caused by an infected dam the design of the study should be altered. It would be useful to choose two groups of animals, one infected and one not infected and monitor all progeny, cows and bulls. These animals would have to be monitored for at least four years, since this is the age most of the animals become positive which makes it a difficult and expensive study. However this would give a more accurate showing of the probability of infection in infected dams versus uninfected dams. To give any information on transmission in utero fetuses should be tested before any contact with environment took place.

Though the results of this study do not show a difference, the numbers found do give rise to some doubt with regard to the current advice given to farmers. It may be needless to cull all progeny of infected dams, since these calves do not seem to have a greater risk on developing MAP as do calves from healthy dams. Also it may just be proof the animals coming from infected dams seem to be less susceptible to MAP making them just the animals we need to diminish this disease. However to make these statements a lot more research has to be done and it will probably take years to find good substantiating evidence to give conclusive advice to farmers concerning MAP.

Conclusions

Concluding from the P-value of the exact test and the confidence interval, the H_0-hypothesis was rejected. In other words, no significant difference can be found in the proportion infected daughters coming from infected dams and the proportion infected daughters coming from uninfected dams. This result can be substantiated with the confidence interval for the odds ratio.

After applying the model and in this way correcting for environmental/animal effects, still no significance was found of the effect of the infection status of the dam on the infection status of the daughter.

Appendix I

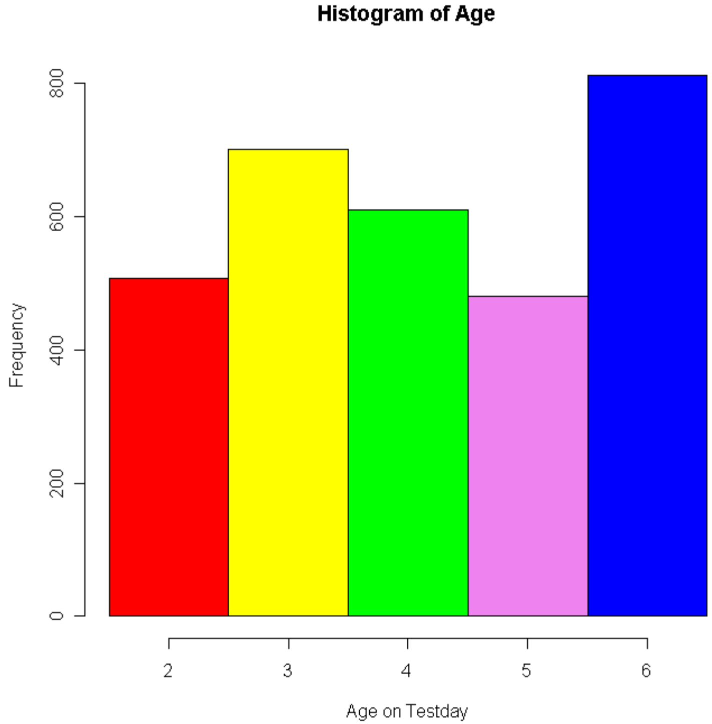
```
#uitslagen inlezen
data <- read.csv ("F:/Onderzoek/R/Uitslagen3.csv", header=TRUE, sep=";",
stringsAsFactors=FALSE, dec=",")
 head (data)

#melkELISA uitfilteren, serumELISA=1
data2 <- data
 data2$sELISA <- ifelse (data2$test == "MilkELISA",0,1)
 data3 <- subset (data2, data2$sELISA >0)
 data4 <- subset (data2, data2$sELISA == 0)
  length (data4$diernummer)
  head (data4)

#wegschrijven naar Excel voor bewerking met SAS (gemiddelde uitslagen per
dier per datum)
excel <- write.table ((data3), file = "F:/Onderzoek/R/sELISA4.csv",
sep=";", col.names=NA)

#output van SAS weer inlezen
data5 <- read.csv ("F:/Onderzoek/R/sas_output(3).csv", header=TRUE,
sep=";", stringsAsFactors=FALSE, dec=",")
data5$attentie <-NULL
data5$COL4 <- NULL
data5$COL5 <- NULL
data5$COL6 <- NULL
data5$dup1 <- NULL
data5$dup2 <- NULL
data5$X_NAME_ <- NULL
head(data5)
length(data5$naam)
summary(data5)
data5$sELISA <- c(1)

#0/1 aan serumuitslag koppelen. Cut-off waarde = 55
v10 <- array(data5$melisa)
uitslaggetal <- array (0,1122)
  for (i in 1:1122)  {if( v10 [i] >= 55) {uitslaggetal[i] <-1}}
data6 <-cbind (data5, uitslaggetal)
  colnames (data6) <-
c("id","mprdatum","naam","ubn","sp","sELISA","uitslaggetal" )
  head (data6)

#0/1 aan melkuitslagkoppelen. Cut-off waarde = 30
v10 <- array(data4$s.p)
uitslaggetal <- array (0,17752)
  for (i in 1:17752)  {if( v10 [i] >= 30) {uitslaggetal[i] <-1}}
data7 <-cbind (data4, uitslaggetal)
 data7$koenaam <- NULL
 data7$mprnummer <-NULL
 data7$elisaplaat <- NULL
 data7$volgnummer <- NULL
 data7$uitslag <- NULL
 data7$koenummer <- NULL
 data7$test <- NULL
  colnames (data7) <- c("naam","ubn","mprdatum", "sp", "id","sELISA",
"uitslaggetal")
  head(data7)

data8<- rbind (data6, data7)
```

```
excel <- write.table ((data8), file = "F:/Onderzoek/R/check.csv", sep=";",
col.names=NA)
summary(data8)

#verwijderen "NL488559841"
data8 <- subset(data8, !(data8$id == "NL488559841" & data8$naam ==
"vdVeen"))
summary(data8)

#subset per bedrijf maken in data9 opslaan
#data9 <- subset(data8, data8$naam == "Uneken")
# length(data9$naam)

#data9 <- subset(data8, data8$naam =="deJong" | data8$naam == "DeJong")
# length(data9$naam)

#data9 <- subset(data8, data8$naam == "Eggenkam" | data8$naam ==
"Eggenkamp")
# length(data9$naam)

#data9 <- subset(data8, data8$naam == "vdVeen" | data8$naam == "vdveen")
# length(data9$naam)

#data9 <- subset(data8, data8$naam == "Dijkstra")
# length(data9$naam)

#data9 <- subset(data8, data8$naam == "Krikke")
# length(data9$naam)

#data9 <- subset(data8, data8$naam == "Neimeije" | data8$naam ==
"Neimeijer")
# length(data9$naam)

#data9 <- subset(data8, data8$naam == "Menken")
# length(data9$naam)

data9 <- data8
 length (data9$naam)

#aantal dieren tellen
 a <- unique(data9$id)
  length (a)

#uitslag serumELISA x uitslag ==> 0/1 voor totale uitslag
data9$totaal_uitslag <- data9$sELISA * data9$uitslaggetal
data9
  x<- tapply (data9$totaal_uitslag,data9$id,sum)
  print(x)
  y <- cbind(x)
  print(y)
  head(y)
  length(y)

#eventueel dam = "." verwijderen
  y2 <- y[c(-1),]
  head(y2)
  length(y2)


#data wegschrijven en opnieuw inlezen
```

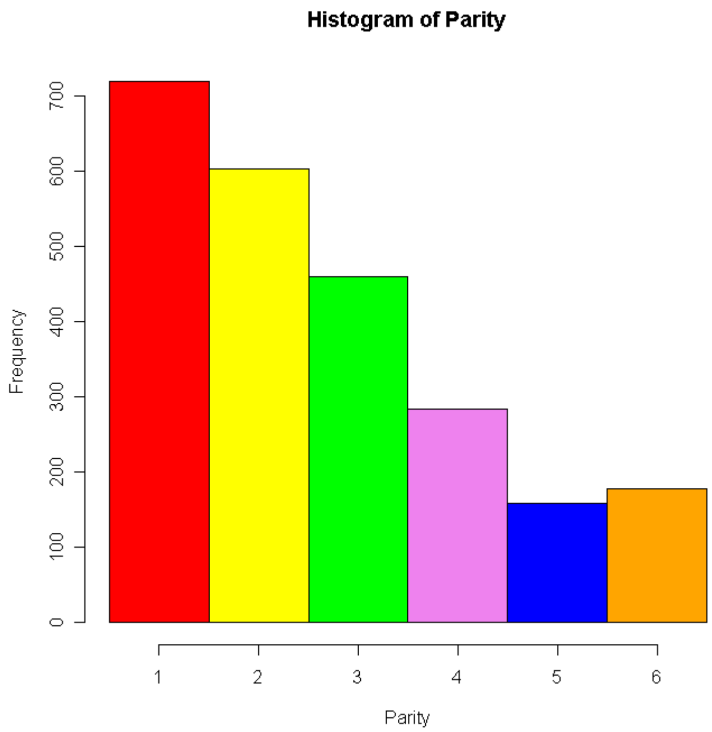
```
excel <- write.table ((y2), file = "F:/Onderzoek/R/Uitslag.csv", sep=";",
col.names=NA)
data_uitslag <- read.csv ("F:/Onderzoek/R/Uitslag.csv", header=TRUE,
sep=";", stringsAsFactors=FALSE, dec=",")
data10 <- data_uitslag
head (data10)

#dier klassificeren als positief of negatief
data10$final <- ifelse(data10$x>0,1,0)

#aantal positieve dieren tellen
m <- subset(data10, data10$final>0)
 length(m$x)

#kolommen hernoemen (id => dam)
 colnames (data10) <- c("dam","som","uitslag")
 head(data10)

#Pedigree inlezen
data_pedigree <- read.csv ("F:/Onderzoek/R/Pedigree_totaal.csv",
header=TRUE, sep=";", stringsAsFactors=FALSE, dec=",")
 head (data_pedigree)

#kolommen verwijderen
 data_pedigree$sire <- NULL
 data_pedigree$date_of_birth <- NULL
 data_pedigree$breed <- NULL
 data_pedigree$gender <- NULL
 data_pedigree$status <- NULL
head (data_pedigree)
data11 <- data_pedigree

#merge data ==> waarbij de testuitslag van de dam achter de PEDIGREE komt
te staan
result <- merge (data10, data11, by = "dam", all=TRUE)
 head(result)

#Naar Excel ter controle van mergen
#excel <- write.table ((result), file = "F:/Onderzoek/R/Controle1.csv",
sep=";", col.names=NA)
#excel <- write.table ((data10), file = "F:/Onderzoek/R/Controle2.csv",
sep=";", col.names=NA)
#controleren: uitslag van de dam met werkelijke uitslag

#kolomnamen opnieuw aanpassen zodat mergen mogelijk is op diernummer
data12 <- result
 colnames (data12) <- c("dam","som","uitslag_dam","animal")
 data12$som <- NULL
 head (data12)
 colnames (data10) <- c("animal","som","uitslag_animal")
 data10$som <-NULL
 head (data10)

#merge data zodat testuitslag van dam achter dier komt te staan
result_totaal <- merge (data10, data12, by = "animal", all=TRUE)
 head (result_totaal)

#data uitschrijven in excel
#excel <- write.table ((result_totaal), file =
"F:/Onderzoek/R/Resultaat_Menken.csv", sep=";", col.names=NA)
```

```
#subset maken: hierbij een uitslag van zowel moeder als dochter in data
aanwezig
 result_positief <- subset(result_totaal, result_totaal$uitslag_animal>=0)
# print(result_positief)
 length(result_positief$uitslag_animal)
 result_alles <- subset (result_positief, result_positief$uitslag_dam >=0)
# print(result_alles)
 length (result_alles$animal)

excel <- write.table ((result_alles), file =
"F:/Onderzoek/R/Resultaat2.csv", sep=";", col.names=NA)
result_alles <-  read.csv ("F:/Onderzoek/R/Resultaat2.csv", header=TRUE,
sep=";", stringsAsFactors=FALSE, dec=",")

#Chi2-test + Fisher's exact
chisq.test (result_alles$uitslag_animal, result_alles$uitslag_dam)$observed
chisq.test (result_alles$uitslag_animal, result_alles$uitslag_dam)$expected
chisq.test (result_alles$uitslag_animal, result_alles$uitslag_dam)
fisher.test (result_alles$uitslag_animal, result_alles$uitslag_dam)

excel <- write.table ((result_alles), file =
"F:/Onderzoek/R/Resultaat2.csv", sep=";", col.names=NA)
```

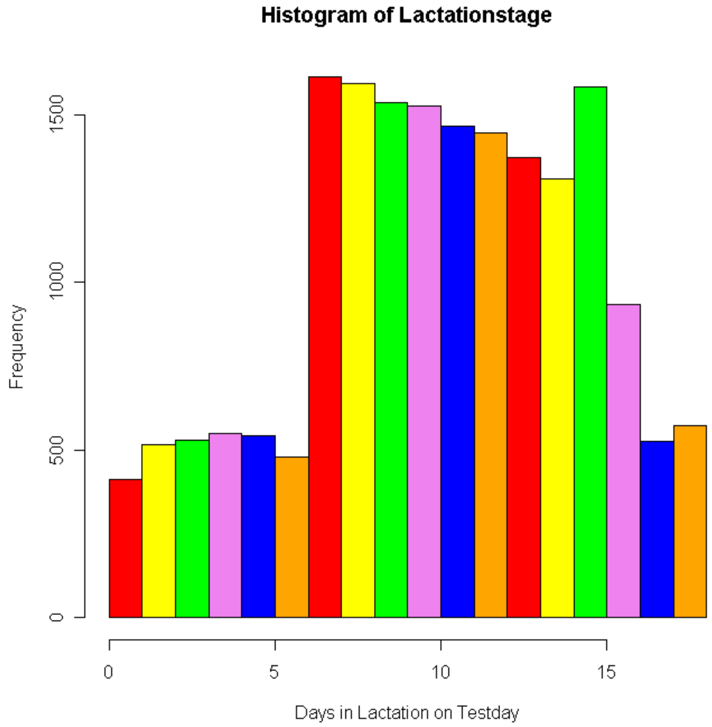
Appendix II



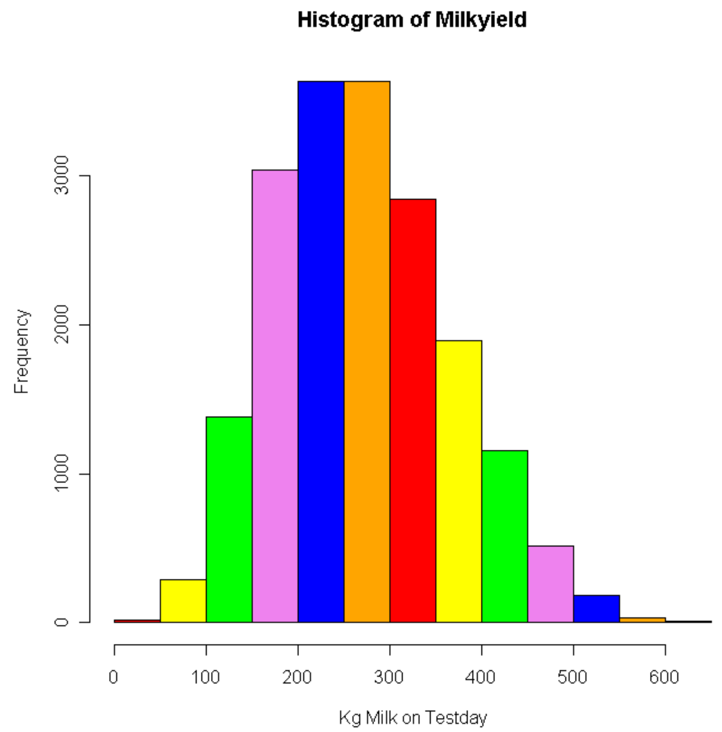
Age	<2	3	4	5	6>
Frequency	508	702	611	481	813



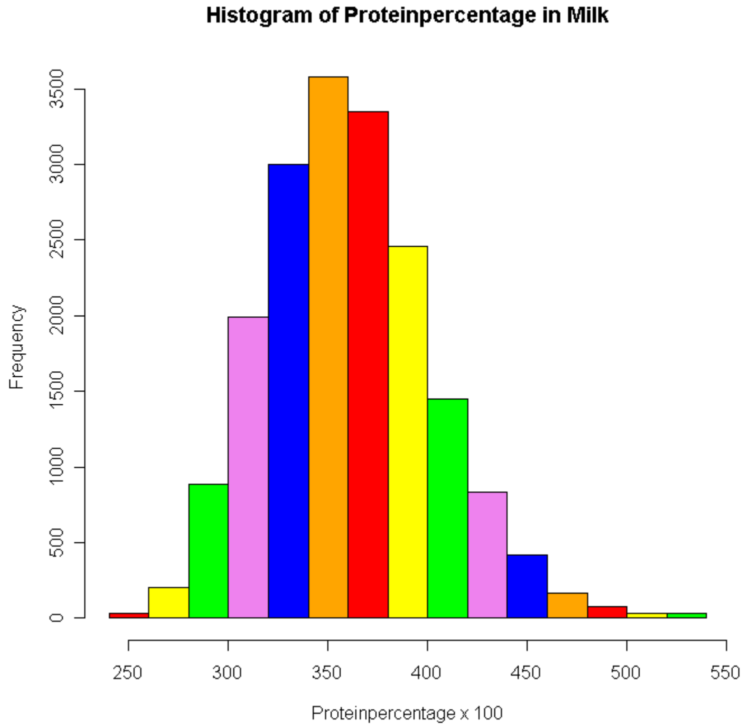
Age	>1	2	3	4	5	6>
Frequency	720	603	460	284	159	178



Class	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
DIM	0-10	10-20	20-30	30-40	40-50	50-60	60-90	90-120	120-150	150-180	180-210	210-240	240-270	270-300	300-350	350-400	400-450	450>
Frequency	413	516	530	550	543	480	1615	1592	1536	1528	1465	1446	1372	1308	1582	933	526	574

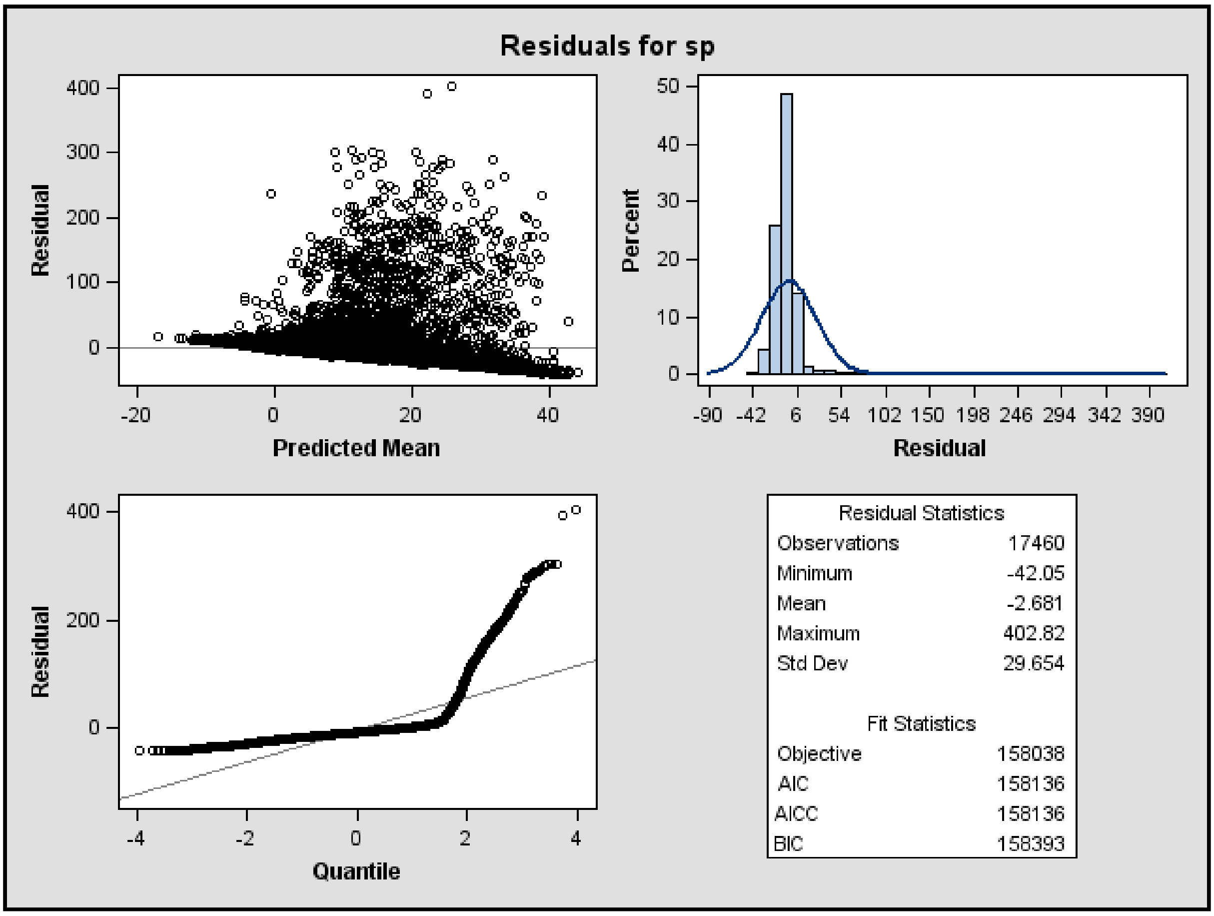


Milk yield	0-50	50-100	100-150	150-200	200-250	250-300	300-350	350-400	400-450	450-500	500-550	550-600	600-650
Frequency	18	286	1381	3040	3639	3637	2848	1897	1157	512	183	30	9

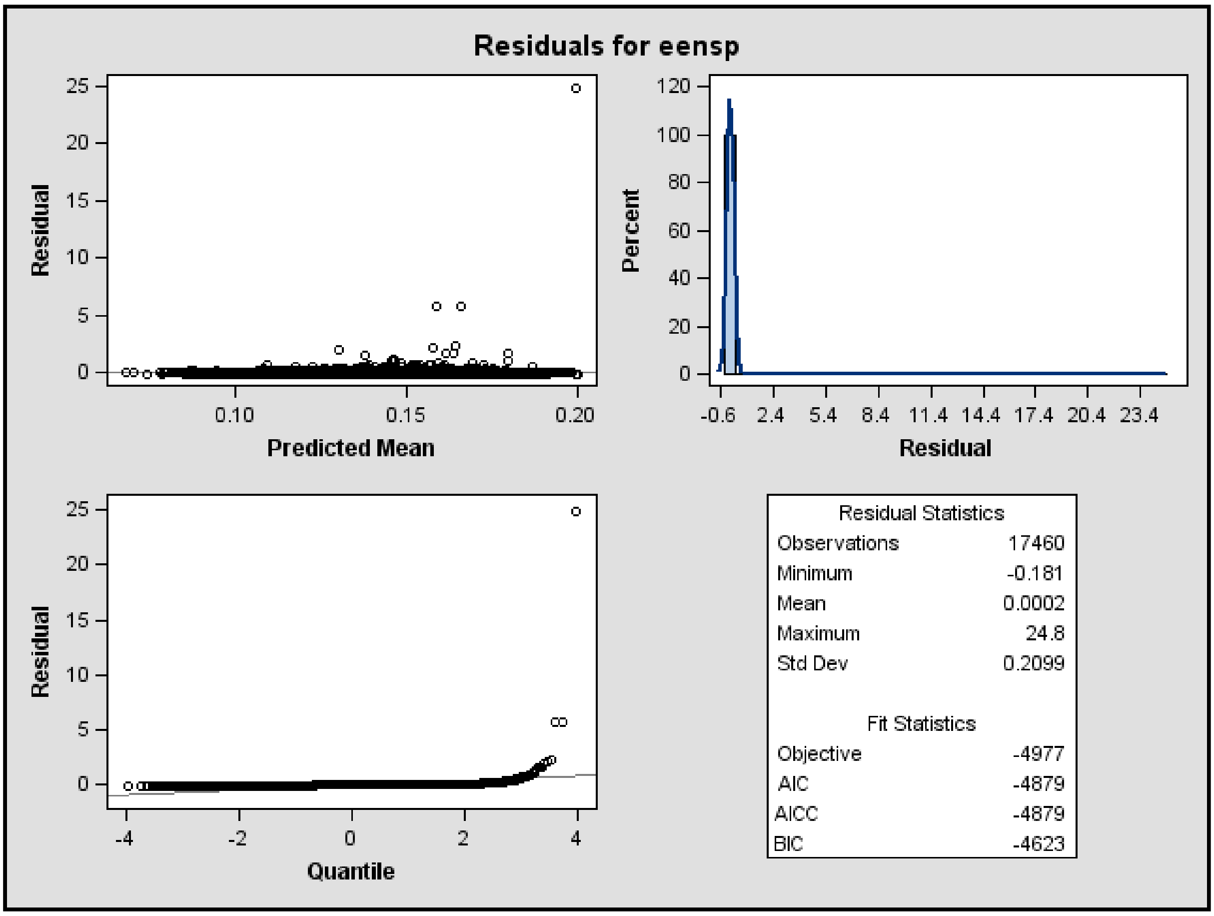


Protein percentage (x100)	<260	260-280	280-300	300-320	320-340	340-360	360-380	380-400	400-420	420-440	440-460	460-480	480-500	500-520	520>
Frequency	31	205	884	1993	3000	3582	3349	2459	1448	831	415	164	80	35	29

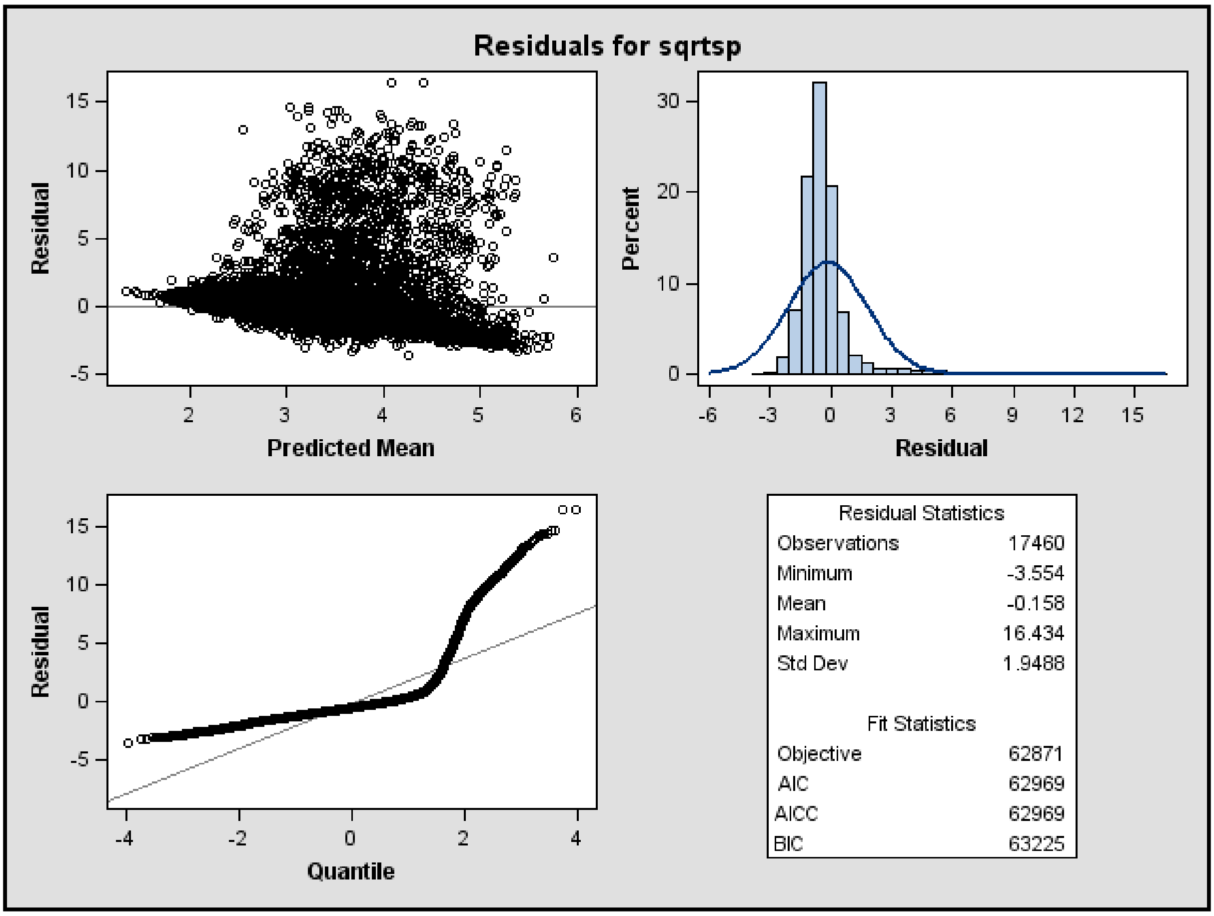
Appendix III



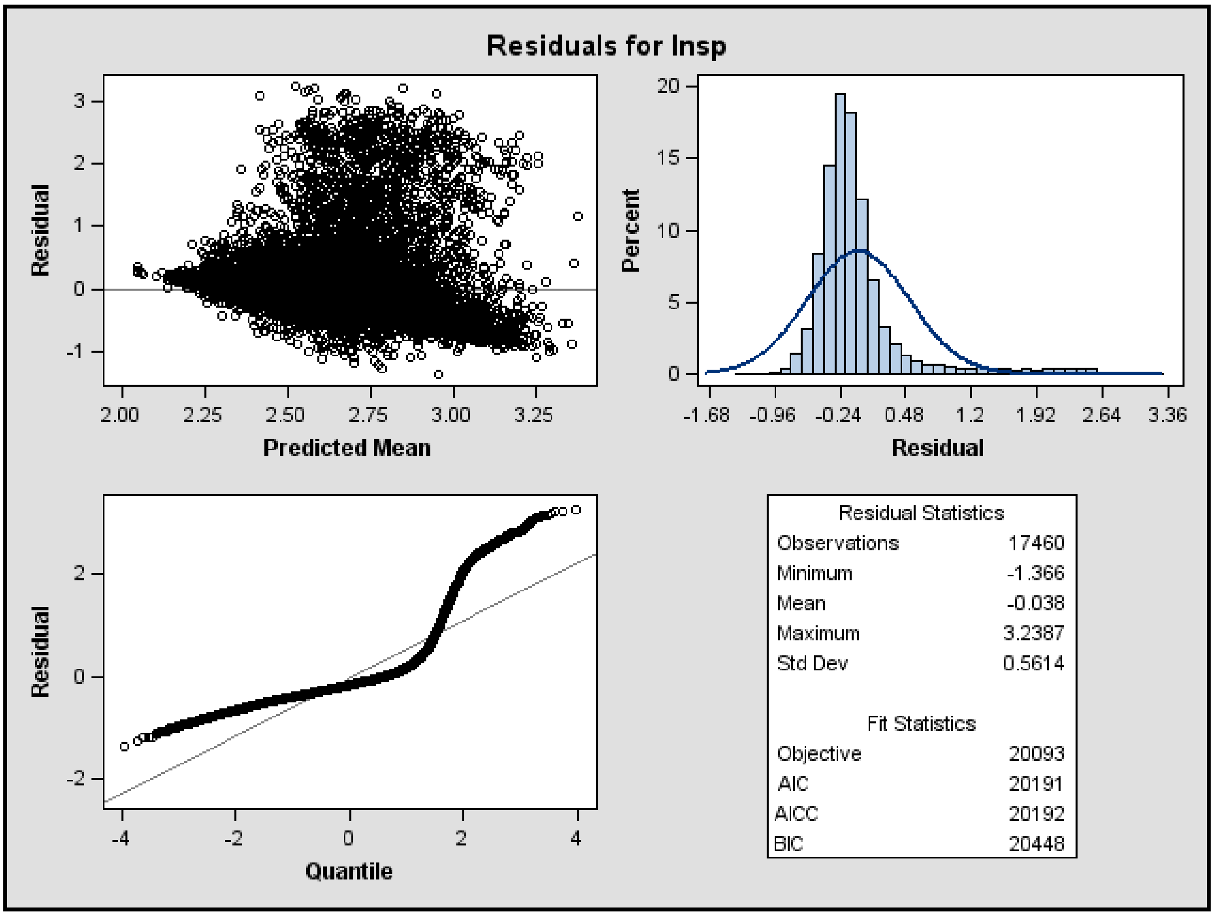
Residualpanel for S/P-ratio



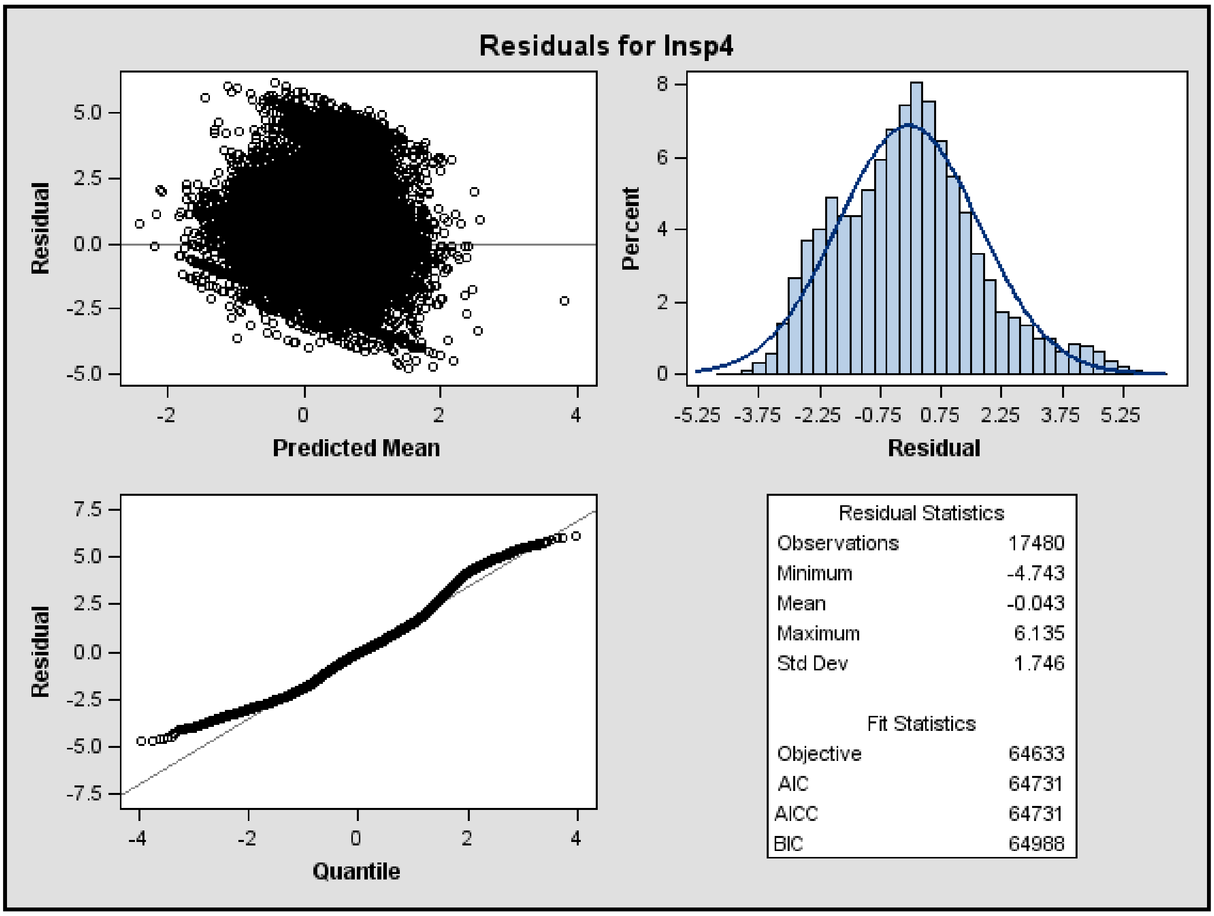
Residual panel for $1/_{S/P-ratio}$



Residual panel for $\sqrt{S/P-ratio}$



Residualpanel for S/P-ratio after adding 10 to each ratio and logtransformation



Residual panel for ln(S/P-ratio) after replacing each negative value with 0.1

Appendix IV

	D		A		P		YOB		UBN		DIM		MY		PP				
Model	**F-Value**	**Pr>F**	**F-Value**	**Pr>F**	**F-Value**	**Pr>F**	**F-Value**	**Pr>F**	**F-Value**	**Pr>F**	**F-Value**	**Pr>F**	**F-Value**	**Pr>F**	**F-Value**	**Pr>F**	**UN**	**RES**	**AIC**
1	0.62	0.5392	1.3	0.2669	18.52	<0.0001	1.77	0.0475	11.75	<0.0001	2.48	0.0007	148.75	<0.0001	3.11	0.078	0.2275	0.1477	20194.1
2	**0.61**	**0.5416**	**-**	**-**	**35.09**	**<0.0001**	**1.69**	**0.063**	**11.69**	**<0.0001**	**2.77**	**0.0001**	**162**	**<0.0001**	**3.28**	**0.0702**	**0.2274**	**0.1478**	**20191.3**
3	0.58	0.5619	21.87	<0.0001	-	-	2.17	0.0106	11.34	<0.0001	3.97	<0.0001	123.99	<0.0001	4.31	0.0379	0.2262	0.1486	20276.4
4	**0.4**	**0.6732**	**1.06**	**0.3761**	**19.46**	**<0.0001**	**-**	**-**	**11.9**	**<0.0001**	**2.52**	**0.0005**	**147.06**	**<0.0001**	**3.2**	**0.0738**	**0.2307**	**0.1478**	**20191.2**
5	1.95	0.1418	1.19	0.3131	17.87	<0.0001	1.86	0.0342	-	-	2.47	0.0007	159.23	<0.0001	3.13	0.077	0.2435	0.1478	20269.5
6	0.6	0.5473	2.56	0.0364	23.62	<0.0001	1.83	0.0375	11.74	<0.0001	-	-	217.54	<0.0001	7.87	0.005	0.2273	0.1481	20202.2
7	0.5	0.6089	4.59	0.001	13.58	<0.0001	1.63	0.0765	13.09	<0.0001	6.5	<0.0001	-	-	30.94	<0.0001	0.2327	0.1488	20340.1
8	0.64	0.5261	1.25	0.289	19.08	<0.0001	1.77	0.0475	11.87	<0.0001	2.53	0.0005	177.67	<0.0001	-	-	0.2251	0.1483	20350.6
9	0.57	0.5672	-	-	-	-	6.42	<0.0001	11.65	<0.0001	3.21	<0.0001	109.27	<0.0001	3.08	0.0792	0.2242	0.1496	20355.6
10	**0.39**	**0.6804**	**-**	**-**	**46.18**	**<0.0001**	**-**	**-**	**11.87**	**<0.0001**	**2.76**	**<0.0001**	**158.72**	**<0.0001**	**3.4**	**0.0654**	**0.2306**	**0.1478**	**20187.5**
11	1.9	0.1491	-	-	34.98	<0.0001	1.8	0.0429	-	-	2.79	0.0001	172.19	<0.0001	3.32	0.0684	0.2433	0.1478	20266.3
12	0.6	0.5505	-	-	36.57	<0.0001	1.66	0.0693	11.72	<0.0001	-	-	218.69	<0.0001	5.89	0.0152	0.2274	0.1482	20204.4
13	0.5	0.6062	-	-	24.52	<0.0001	1.42	0.1504	12.99	<0.0001	6.09	<0.0001	-	-	32.31	<0.0001	0.2325	0.149	20350.5
14	0.64	0.5283	-	-	36.05	<0.0001	1.69	0.0627	11.83	<0.0001	2.74	0.0001	192.21	<0.0001	-	-	0.2250	0.1484	20347.5
15*	1.6	0.2060	-	-	13.67	<0.0001	3.64	0.0003	3.37	0.0007	3.46	<0.0001	47.02	<0.0001	8.95	0.0028	0.1605	0.1163	5601.5

Type III tests of fixed effects, for each model the F-values and the corresponding P-value are viewed. Also the AIC and the univariate (UN) and residual (RES) covariant components are described.

Model 15 represents the data set with only known infection status

Appendix V

Effect	DAM	P	YOB	UBN	DIM	Estimate	Standard Error	DF	t/Value	Pr:|t|
Intercept						2.7649	0.1042	17E3	26.54	<.0001
DAM	0					0.02035	0.03398	17E3	0.60	0.5493
DAM	1					0.07353	0.07108	17E3	1.03	0.3010
DAM	2					0	.	.	.	.
P		1				-0.3058	0.04605	17E3	-6.64	<.0001
P		2				-0.2093	0.04373	17E3	-4.79	<.0001
P		3				-0.07689	0.04121	17E3	-1.87	0.0621
P		4				-0.03488	0.03775	17E3	-0.92	0.3554
P		5				-0.04489	0.03055	17E3	-1.47	0.1417
P		6				0	.	.	.	.
YOB			1997			-0.2002	0.3904	17E3	-0.51	0.6081
YOB			1998			0.1831	0.2141	17E3	0.86	0.3924
YOB			1999			-0.1899	0.2042	17E3	-0.93	0.3525
YOB			2000			0.004936	0.1335	17E3	0.04	0.9705
YOB			2001			-0.05003	0.1111	17E3	-0.45	0.6524
YOB			2002			0.1192	0.09466	17E3	1.26	0.2079
YOB			2003			0.1690	0.08118	17E3	2.08	0.0374
YOB			2004			0.1156	0.07240	17E3	1.60	0.1104
YOB			2005			0.1407	0.06699	17E3	2.10	0.0357
YOB			2006			0.1611	0.06237	17E3	2.58	0.0098
YOB			2007			0.1633	0.06018	17E3	2.71	0.0067
YOB			2008			0.06054	0.05855	17E3	1.03	0.3012
YOB			2009			0	.	.	.	.
UBN				A		0.4456	0.06015	17E3	7.41	<.0001
UBN				B		0.09122	0.05623	17E3	1.62	0.1048
UBN				C		0.1269	0.05982	17E3	2.12	0.0339
UBN				D		0.1078	0.05716	17E3	1.89	0.0593
UBN				E		0.09787	0.05471	17E3	1.79	0.0737
UBN				F		0.2183	0.05947	17E3	3.67	0.0002
UBN				G		0.03062	0.02165	17E3	1.41	0.1571
UBN				H		-0.03488	0.05698	17E3	-0.61	0.5405
UBN				I		0	.	.	.	.
DIM					1	-0.00702	0.02979	17E3	-0.24	0.8138
DIM					2	-0.03435	0.03019	17E3	-1.14	0.2553
DIM					3	-0.04485	0.03105	17E3	-1.44	0.1486
DIM					4	-0.02339	0.03123	17E3	-0.75	0.4538
DIM					5	-0.03894	0.03163	17E3	-1.23	0.2183
DIM					6	-0.02698	0.03200	17E3	-0.84	0.3990
DIM					7	-0.04905	0.02699	17E3	-1.82	0.0692
DIM					8	-0.05498	0.02603	17E3	-2.11	0.0347
DIM					9	-0.07377	0.02532	17E3	-2.91	0.0036
DIM					10	-0.07363	0.02466	17E3	-2.99	0.0028
DIM					11	-0.07841	0.02425	17E3	-3.23	0.0012
DIM					12	-0.09549	0.02386	17E3	-4.00	<.0001
DIM					13	-0.05666	0.02348	17E3	-2.41	0.0158
DIM					14	-0.06171	0.02318	17E3	-2.66	0.0078
DIM					15	-0.05748	0.02230	17E3	-2.58	0.0100
DIM					16	-0.04228	0.02349	17E3	-1.80	0.0719
DIM					17	-0.05559	0.02575	17E3	-2.16	0.0309
DIM					18	0	.	.	.	.
MY						-0.00086	0.000068	17E3	-12.73	<.0001
PP						0.000240	0.000133	17E3	1.81	0.0702

Estimates for model 2

Effect	DAM	P	YOB	UBN	DIM	Estimate	Standard Error	DF	t/Value	Pr:|t|
DAM	0					2.6511	0.04839	17E3	54.79	<.0001
DAM	1					2.7043	0.07951	17E3	34.01	<.0001
DAM	2					2.6307	0.04075	17E3	64.56	<.0001
P		1				2.4682	0.05193	17E3	47.53	<.0001
P		2				2.5647	0.05077	17E3	50.52	<.0001
P		3				2.6971	0.04989	17E3	54.06	<.0001
P		4				2.7391	0.04936	17E3	55.49	<.0001
P		5				2.7291	0.04948	17E3	55.15	<.0001
P		6				2.7740	0.05122	17E3	54.16	<.0001
YOB			1997			2.4097	0.3855	17E3	6.25	<.0001
YOB			1998			2.7930	0.2057	17E3	13.58	<.0001
YOB			1999			2.4200	0.1948	17E3	12.42	<.0001
YOB			2000			2.6149	0.1195	17E3	21.88	<.0001
YOB			2001			2.5599	0.09428	17E3	27.15	<.0001
YOB			2002			2.7291	0.07538	17E3	36.20	<.0001
YOB			2003			2.7789	0.06081	17E3	45.70	<.0001
YOB			2004			2.7255	0.05072	17E3	53.73	<.0001
YOB			2005			2.7506	0.04604	17E3	59.75	<.0001
YOB			2006			2.7710	0.04092	17E3	67.72	<.0001
YOB			2007			2.7733	0.04071	17E3	68.13	<.0001
YOB			2008			2.6705	0.04051	17E3	65.92	<.0001
YOB			2009			2.6099	0.05812	17E3	44.90	<.0001
UBN				A		2.9872	0.06072	17E3	49.20	<.0001
UBN				B		2.6329	0.05768	17E3	45.65	<.0001
UBN				C		2.6686	0.06075	17E3	43.93	<.0001
UBN				D		2.6495	0.05821	17E3	45.52	<.0001
UBN				E		2.6395	0.05658	17E3	46.65	<.0001
UBN				F		2.7599	0.06073	17E3	45.45	<.0001
UBN				G		2.5723	0.06006	17E3	42.83	<.0001
UBN				H		2.5068	0.05915	17E3	42.38	<.0001
UBN				I		2.5416	0.05997	17E3	42.38	<.0001
DIM					1	2.7036	0.05117	17E3	52.83	<.0001
DIM					2	2.6763	0.05015	17E3	53.36	<.0001
DIM					3	2.6658	0.05024	17E3	53.06	<.0001
DIM					4	2.6872	0.05018	17E3	53.55	<.0001
DIM					5	2.6717	0.05034	17E3	53.08	<.0001
DIM					6	2.6836	0.05066	17E3	52.97	<.0001
DIM					7	2.6616	0.04792	17E3	55.54	<.0001
DIM					8	2.6556	0.04776	17E3	55.61	<.0001
DIM					9	2.6368	0.04770	17E3	55.28	<.0001
DIM					10	2.6370	0.04761	17E3	55.39	<.0001
DIM					11	2.6322	0.04764	17E3	55.25	<.0001
DIM					12	2.6151	0.04770	17E3	54.83	<.0001
DIM					13	2.6540	0.04779	17E3	55.53	<.0001
DIM					14	2.6489	0.04794	17E3	55.26	<.0001
DIM					15	2.6531	0.04782	17E3	55.48	<.0001
DIM					16	2.6683	0.04888	17E3	54.59	<.0001
DIM					17	2.6550	0.05065	17E3	52.42	<.0001
DIM					18	2.7106	0.05116	17E3	52.99	<.0001

Least square means for model 2

Appendix VI

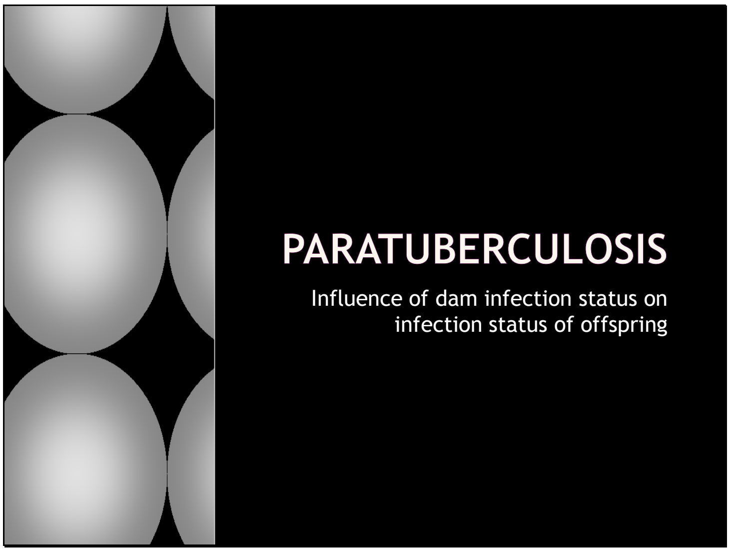
Effect	DAM	P	YOB	UBN	DIM	Estimate	Standard Error	DF	t/Value	Pr:|t|
Intercept						2.5151	0.1885	6044	13.34	<.0001
DAM	0					-0.07924	0.06265	6044	-1.26	0.2060
DAM	1					0	.	.	.	.
P		1				-0.06897	0.1322	6044	-0.52	0.6018
P		2				-0.01211	0.1304	6044	-0.09	0.9260
P		3				0.1386	0.1285	6044	1.08	0.2805
P		4				0.1021	0.1244	6044	0.82	0.4119
P		5				0.03243	0.1177	6044	0.28	0.7829
P		6				0	.	.	.	.
YOB			2000			2.0405	0.4510	6044	4.52	<.0001
YOB			2002			0.1748	0.4354	6044	0.40	0.6881
YOB			2003			0.2219	0.1390	6044	1.60	0.1105
YOB			2004			0.09463	0.1150	6044	0.82	0.4105
YOB			2005			0.1654	0.09520	6044	1.74	0.0823
YOB			2006			0.1840	0.07262	6044	2.53	0.0113
YOB			2007			0.1587	0.06497	6044	2.44	0.0146
YOB			2008			0.05682	0.05848	6044	0.97	0.3313
YOB			2009			0	.	.	.	.
UBN				A		0.3416	0.08690	6044	3.93	<.0001
UBN				B		0.09824	0.06859	6044	1.43	0.1521
UBN				C		0.07901	0.07311	6044	1.08	0.2799
UBN				D		0.08901	0.08153	6044	1.09	0.2749
UBN				E		0.1682	0.08062	6044	2.09	0.0370
UBN				F		0.2753	0.08176	6044	3.37	0.0008
UBN				G		0.003032	0.02993	6044	0.10	0.9193
UBN				H		0.02118	0.07210	6044	0.29	0.7689
UBN				I		0	.	.	.	.
DIM					1	-0.05320	0.04641	6044	-1.15	0.2517
DIM					2	-0.04745	0.04709	6044	-1.01	0.3136
DIM					3	-0.06338	0.04860	6044	-1.30	0.1922
DIM					4	-0.06164	0.04955	6044	-1.24	0.2136
DIM					5	-0.09458	0.04964	6044	-1.91	0.0568
DIM					6	-0.05021	0.04987	6044	-1.01	0.3140
DIM					7	-0.08793	0.04363	6044	-2.02	0.0439
DIM					8	-0.1073	0.04223	6044	-2.54	0.0111
DIM					9	-0.1471	0.04129	6044	-3.56	0.0004
DIM					10	-0.1284	0.04054	6044	-3.17	0.0015
DIM					11	-0.1536	0.03996	6044	-3.84	0.0001
DIM					12	-0.1563	0.03953	6044	-3.95	<.0001
DIM					13	-0.1355	0.03913	6044	-3.46	0.0005
DIM					14	-0.1299	0.03890	6044	-3.34	0.0008
DIM					15	-0.1361	0.03779	6044	-3.60	0.0003
DIM					16	-0.1325	0.03986	6044	-3.32	0.0009
DIM					17	-0.1615	0.04434	6044	-3.64	0.0003
DIM					18	0	.	.	.	.
MY						-0.00072	0.000105	6044	-6.86	<.0001
PP						0.000621	0.000208	6044	2.99	0.0028

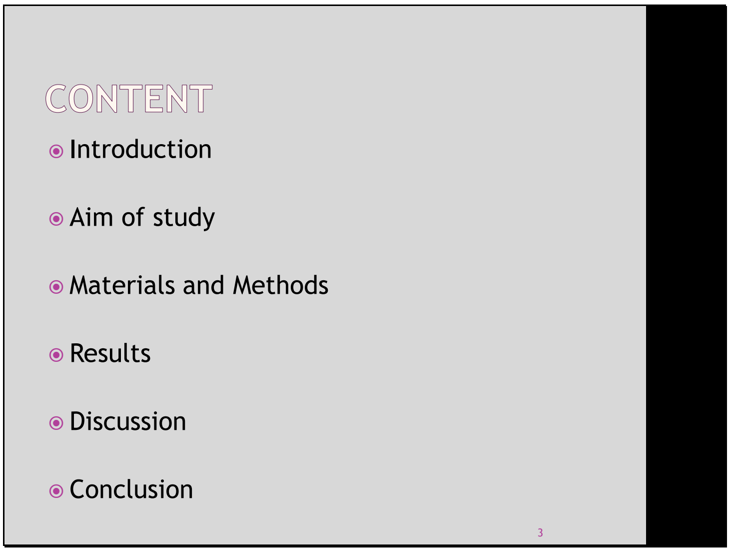
Estimates for model 15

Effect	DAM	P	YOB	UBN	DIM	Estimate	Standard Error	DF	t/Value	Pr:|t|
DAM	0					2.8538	0.07020	6044	40.65	<.0001
DAM	1					2.9331	0.08970	6044	32.70	<.0001
P		1				2.7925	0.08277	6044	33.74	<.0001
P		2				2.8493	0.08169	6044	34.88	<.0001
P		3				3.0001	0.08139	6044	36.86	<.0001
P		4				2.9635	0.08232	6044	36.00	<.0001
P		5				2.8939	0.08767	6044	33.01	<.0001
P		6				2.8614	0.1218	6044	23.48	<.0001
YOB			2000			4.5899	0.4403	6044	10.42	<.0001
YOB			2002			2.7242	0.4247	6044	6.41	<.0001
YOB			2003			2.7712	0.1241	6044	22.33	<.0001
YOB			2004			2.6440	0.1026	6044	25.76	<.0001
YOB			2005			2.7148	0.08657	6044	31.36	<.0001
YOB			2006			2.7334	0.06161	6044	44.37	<.0001
YOB			2007			2.7081	0.05833	6044	46.43	<.0001
YOB			2008			2.6062	0.05397	6044	48.29	<.0001
YOB			2009			2.5493	0.06767	6044	37.67	<.0001
UBN				A		3.1155	0.09702	6044	32.11	<.0001
UBN				B		2.8722	0.08619	6044	33.32	<.0001
UBN				C		2.8529	0.08949	6044	31.88	<.0001
UBN				D		2.8629	0.09434	6044	30.35	<.0001
UBN				E		2.9421	0.09502	6044	30.96	<.0001
UBN				F		3.0492	0.09465	6044	32.22	<.0001
UBN				G		2.7770	0.08662	6044	32.06	<.0001
UBN				H		2.7951	0.09056	6044	30.87	<.0001
UBN				I		2.7739	0.08514	6044	32.58	<.0001
DIM					1	2.9428	0.07975	6044	36.90	<.0001
DIM					2	2.9486	0.07842	6044	37.60	<.0001
DIM					3	2.9326	0.07871	6044	37.26	<.0001
DIM					4	2.9344	0.07928	6044	37.01	<.0001
DIM					5	2.9014	0.07908	6044	36.69	<.0001
DIM					6	2.9458	0.07948	6044	37.06	<.0001
DIM					7	2.9081	0.07603	6044	38.25	<.0001
DIM					8	2.8888	0.07575	6044	38.13	<.0001
DIM					9	2.8489	0.07569	6044	37.64	<.0001
DIM					10	2.8676	0.07563	6044	37.92	<.0001
DIM					11	2.8425	0.07559	6044	37.60	<.0001
DIM					12	2.8398	0.07579	6044	37.47	<.0001
DIM					13	2.8605	0.07593	6044	37.67	<.0001
DIM					14	2.8662	0.07624	6044	37.59	<.0001
DIM					15	2.8599	0.07627	6044	37.50	<.0001
DIM					16	2.8635	0.07805	6044	36.69	<.0001
DIM					17	2.8346	0.08132	6044	34.86	<.0001
DIM					18	2.9960	0.08252	6044	36.31	<.0001

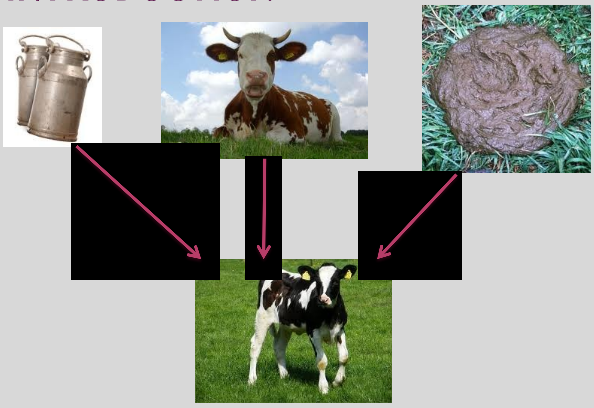
Least square means for model 15

Appendix VII





INTRODUCTION



4

AIM STUDY

- Knowledge on vertical transmission
- By using
  - 2x2 table
  - Mixed model

5

DATA FOR 2X2 TABLE

- Data MESDAG
  - 1378 cows
  - 8 farms
  - 129 positive animals
- Pedigree
  - 13097 cows
- Combinations mother/daughter
  - 486 combinations

	Milk	Serum
Negative	<20%	<45%
Questionable	20-30%	45-55%
Positive	>30%	>55%

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MATERIALS & METHODS 2X2 TABLE

$$H_0 = p_1 = p_2$$
$$H_1 = p_1 \neq p_2$$

Observed — Dams

Animals		Infected	Uninfected	Total
	Infected	a	b	a + b
	Uninfected	c	d	c + d
	Total	a + b	b + d	a + b + c + d

- Fisher's exact test + Confidence interval
- Odds ratio + Confidence interval

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RESULTS 2X2 TABLE

Observed — Dams

Animals		Infected	Uninfected	Total
	Infected	7	25	32
	Uninfected	49	405	454
	Total	56	430	486

$$p_1 = \frac{7}{(7+49)} = 0.125 \qquad p_2 = \frac{25}{(25+405)} = 0.058$$

P-value: 0.079, 95%-CI = (-0.023; 0.157)

OR: 2.309, 95%-CI = (0.801; 5.868)

8

DATA MODEL

- Data MESDAG
  - Just milk
  - 19592 observations
  - 1391 animals
- MPR data
  - 17460 observations
  - 1388 cows
- Infection status from 2x2 table
  - 484 known: 54 positive, 430 negative,
  - 904 missing

9

MATERIALS & METHODS MODEL

- Model
  - Log transformated Y-variable: s/p ratio
  - Fixed effects: Parity (P), Herd (UBN), Birth year (YOB), Lactation stage (DIM), Milk Yield (MY), Protein percentage (PP), Dam infection status (DAM)
  - Random effects: animal, residuals

$$\ln(Y_{jklmnopq} + 10) = P_i + UBN_j + YOB_k + DIM_l + MY_m + PP_n + DAM_o + animal_p + e_{jklmnopq}$$

- Fitted using AIC

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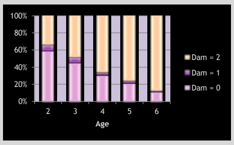
RESULTS MODEL

Variable	F-value	P-value
DAM	0.61	0.5416
P	35.09	<0.0001
YOB	1.69	0.063
UBN	11.69	<0.0001
DIM	2.77	0.0001
MY	162	<0.0001
PP	3.28	0.0702

Variable	F-value	P-value
DAM	1.9	0.1491
P	34.98	<0.0001
YOB	1.8	0.0429
UBN	-	-
DIM	2.79	0.0001
MY	172.19	<0.0001
PP	3.32	0.0684



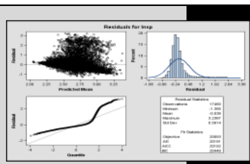
11

RESULTS MODEL (2)

Variable	F-value	P-value
DAM	0.61	0.5416
P	35.09	<0.0001
YOB	1.69	0.063
UBN	11.69	<0.0001
DIM	2.77	0.0001
MY	162	<0.0001
PP	3.28	0.0702

Variable	F-value	P-value
DAM	1.6	0.2060
P	13.67	<0.0001
YOB	3.64	0.0003
UBN	3.37	0.0007
DIM	3.46	<0.0001
MY	47.02	<0.0001
PP	8.95	0.0028

12



DISCUSSION

- No information in utero infection
- Measure application
- Protective vs. negative effect
- Missed positives
- Skewness
- Cull effect
- No significant effect: more infected animals needed and longitudinal data longer time span
- However: suggestive findings

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CONCLUSION

- H_0-hypothesis rejected

- No significance found of effect of infection status dam on infection status daughter

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QUESTIONS?

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