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## STATISTICAL ANALYSIS OF PIGLET STILLBIRTH

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### INTRODUCTION

Up to 8% of newborn piglets are stillborn, predominantly as a result of perinatal asphyxiation experienced in uterus or during delivery. This proportion tends to increase as a correlated response to selection for litter size. Limiting or reducing the number of stillbirths requires its major determinants to be investigated. Genetic influences on stillbirth have been reported: Meishan sows are highly prolific animals with low stillbirth (Bidanel *et al.*, 1989). Stillbirth is not normally distributed and has complex relationships with its main factors of variation, but has so far seldom been analysed using proper statistical methodologies. Generalized estimating equations (GEE, Liang and Zeger 1986, Zeger and Liang, 1986) are popular methods to handle discrete data and take into account simple structures of correlations, such as correlations due to the common environment of birth litter. The objective of the present study was to analyse piglet stillbirth with GEE while avoiding difficulties associated to the low occurrence of stillbirth in Meishan sows and the use of field data.

### MATERIAL AND METHODS

**Animals and data.** Several litter characteristics at birth, including the number and weight of piglets born alive and stillborn, were recorded on 969 litters farrowed by 511 sows out of four different genetic types: Large White (**LW** – 651 litters), Meishan (**MS** – 63 litters), Laconie male line (**LA** – 173 litters – a synthetic line based on Hampshire, Pietrain and Large White breeds) and F1 Duroc x Large White (**DU x LW** – 82 litters). The data were collected in an INRA experimental herd from 1999 to 2003. Sows were housed in individual crates with slatted-floor for parturition and during lactation. Farrowing was induced with prostaglandin on the 113<sup>th</sup> day of gestation. The need for farrowing assistance resulted from specific indications, e.g., a lengthy interval between successive births and/or a lack of contractions. Newborn piglets were individually weighed and sexed within 24 hours after farrowing.

**Statistical analyses.** A large number of potential factors of variation in stillbirth were investigated, i.e., sow genetic type (**GT**), parity (**P**), birth assistance (**BA**), and sex (**SEX**) as fixed effects; gestation length (**GEST**), total number born (**TNB**) and birth weight as covariates; sow permanent environment and/or birth litter as random effects. The effect of birth weight was investigated using the difference (**DBW**) from the within-litter Mean Birth Weight (**MBW**). Birth assistance was considered as a binary trait (0 = no assistance / 1 = one or several assistances). The parity effect included five categories (1, 2, 3, 4,  $\geq 5$ ).

Statistical analyses of piglet stillbirth probability were carried out considering all fixed effects and their first order interactions, selected according to F statistics using a backward elimination with correction for overdispersion, applied to a generalised linear model using the GENMOD procedure of the SAS software (SAS Institute, 2001). A binomial distribution was preferred to a Poisson distribution for stillbirth based on the results of a comparison of deviance and Deviance Information Criterion (DIC) from a random logistic regression in Bayesian

Hierarchical Models performed via the Winbugs software (Spiegelhalter et al., 2003). Bayesian selection additionally indicated that the model with litter as a single random effect was satisfactory to take into account correlations among piglets. Then, piglet stillbirth probability was modelled with Generalized Estimating Equations (GEE) that take into account correlations among piglets within-litter. Classical GEE analyses (Liang and Zeger, 1986; Zeger and Liang, 1986) were performed with the GENMOD procedure; they allowed the contribution of each effect to the variance reduction to be evaluated using a stepwise procedure. The classical GEE estimator for both regression and nuisance parameters can be highly influenced by deviating data points. A robust version of GEE (Cantoni, 2004) was hence used with the S-PLUS software (Insightful Corp., 2004) and routines available from E. Cantoni. The robust GEE procedure is a tool allowing estimators and test statistics that are stable in a neighborhood of the postulated model to be obtained and can be seen as a weighted version of the standard GEE equations. Inspection of the weights from a fitted robust GEE model allows departing observations, i.e., those that have been given small weights, to be identified. A robust binary GEE model including GT, parity and BA as fixed effects was fitted, and compared to a standard GEE. Characterization of the subpopulation of down-weighted observations from this model was carried out using the SAS GLM procedure (SAS Institute, 2001). It allowed separation between the space containing observations of value 0 (i.e., piglet survival at birth) and the space containing observations of value 1 (i.e., piglet stillbirth) to be suspected. Given that a complete separation between the two spaces would imply nonexistence of the estimator, the methodology of Christmann and Rousseeuw (2001) was used (package ncomplete from R software, A. Christmann) to compute the overlap, i.e., the smallest number of observations whose removal yielded complete separation.

## RESULTS AND DISCUSSION

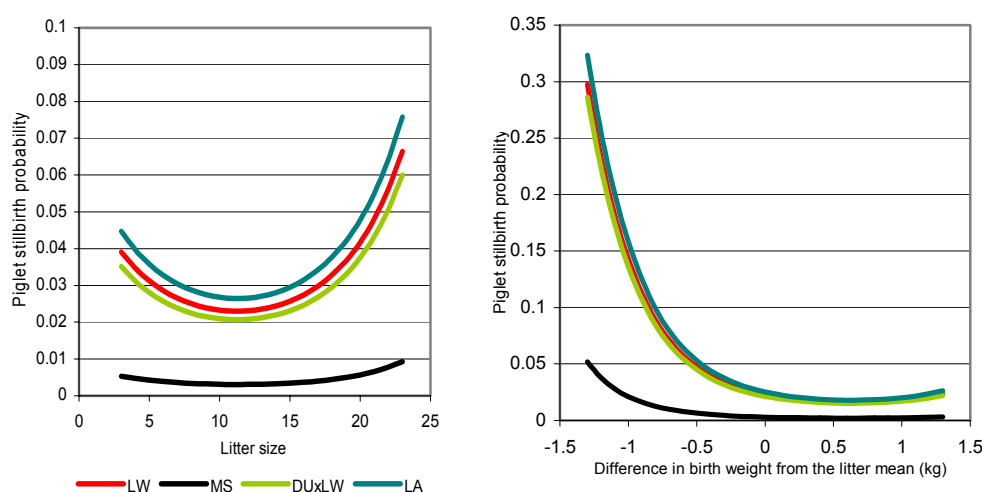
**Factors of variation of stillbirth.** The reduction in residual sum of squares due to successive addition of each explanatory variable demonstrated that the difference in birth weight (DBW) from the mean of the litter was the main determinant of piglet survival (+2.25% in variance reduction), followed by SEX, sow GT and parity (1.01%, 0.94% and 0.50%, respectively), and DBW<sup>2</sup> (0.23%). The remaining effects had lower contributions (<0.21%). Results from GEE analyses also demonstrated the strong influences of GT, SEX and DBW on stillbirth probability (Table 1). Piglets from MS sows had an 86% lower stillbirth probability than piglets born from LW sows, in agreement with Bidanel et al. (1989). Stillbirth probability was 1.6 times higher in the fifth (or greater) parity. Male piglets had a 1.8 times higher stillbirth probability than female piglets. Piglets from litters with assistance had a 1.4 times higher stillbirth probability than piglets from litters without birth assistance. Litter size had a non linear effect on stillbirth probability, except for litters from Meishan sows, where it remained almost constant (figure 1a). In the three other genetic types, piglets from small and large litters were more susceptible to die since large litters are associated with a longer farrowing duration and a higher risk of hypoxia (Herpin et al., 2001) and small litters might be observed in sows with physiological inabilities or difficulties to have a normal gestation. The risk of stillbirth grew rapidly when piglet weight fell below litter average (figure 1b) (Roche, 2003).

**Table 1. GEE estimates of global tests, level effects, and contributions to the reduction of deviance (R) for stillbirth probability.**

	Global test ( $\chi^2$ ) <sup>A</sup>	Level effect (SE) <sup>B</sup>	R (%)
Intercept		3.25 (6.09)	
GT DU x LW	44.08 ***	-0.06 (0.19)	0.94
GT MS		-2.05 (0.42) ***	
GT LA		0.12 (0.14)	
P	11.06 *		0.50
SEX	47.37 ***	0.64 (0.09) ***	1.01
GEST	0.54 ns	-0.04 (0.05)	0.06
BA	4.04 *	0.38 (0.17) *	0.15
TNB	4.94 *	-0.22 (0.08) **	0.12
TNB <sup>2</sup>	5.54 *	0.01 (0.00) **	0.21
MBW	-1.07 ns	-0.99 (0.92)	0.25
MBW <sup>2</sup>	0.17 ns	0.11 (0.27)	0.007
DBW	52.75 ***	-1.11 (0.21) ***	2.36
DBW <sup>2</sup>	3.58 °	0.88 (0.38) *	0.23

<sup>A</sup> Levels of significance for  $\chi^2$ -statistics: ns: not significant; ° p<0.10; \* p<0.05; \*\* p<0.01; \*\*\* p<0.001. <sup>B</sup> Figures correspond to the log odds ratio. Then, odds ratio can be obtained with an exponential transformation. The different parity levels were not detailed here. Levels of significance for z-statistics were the same as in footnote 1. For continuous traits, the sign of the level effect indicates the direction of the relationship with stillbirth probability. Example: a one day longer gestation was associated with a 4% lower stillbirth probability.

**Methodological aspects.** The results obtained with the robust GEE approach were very similar to standard GEE results. An analysis of variance of the weights attributed to the data demonstrated that points with weights < 1 were allocated to nearly all stillborn piglets (562 among 568); thus, we must be cautious in interpreting the results due to the unbalanced experimental design. Weights allocation was in particular dependant on sow GT (p<0.0001) with lower weights attributed to litters from Meishan sows: their low stillbirth rate is likely to have promoted this variable as a cause of separability. Moreover, the overlap between the y=0 and y=1 observations only involved 8 of the 11735 observations. However, the hidden logistic regression approach of Rousseeuw and Christmann (2003) provided similar estimates to those obtained from the robust GEE procedure. The separation issue arises only with binary regression, whereas the procedure of Rousseeuw and Christmann (2003) does not suffer from this problem. Given that we have obtained consistent results throughout the different approaches, we can be reasonably confident about them.



**Figure 1. Patterns of litter size effect (left) and difference in birth weight from the litter mean effect (right) on piglet stillbirth probability according to sow genetic type.**

## CONCLUSION

The robustness of a statistical analysis is an important issue, often neglected. Using complementary statistical methodologies in the analysis of complex unbalanced data is a way to address this problem. Even if marginal, GEE are adequate methods to model piglet stillbirth: estimates obtained in a parallel study with exact methods were in line with the present results. The important role of birth weight on stillbirth probability was pointed out: selecting for decreased weight variability might be a way to reduce piglet mortality. Meishan sows are of interest to understand genetic and physiological causes of variation in stillbirth.

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