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Graphical approaches to support the analysis of linear-multilevel models of lamb pre-weaning growth in Kolda (Senegal)

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Abstract

Linear-multilevel models (LMM) are mixed-effects models in which several levels of grouping may be specified (village, herd, animal, . . .). This study highlighted the usefulness of graphical methocis in their analysis through: (1) the choice of the fixed and random effects and their structure, (2) the assessment of goodness-of-fit and (3) distributional assumptions for random effects and residuals.

An LMM was developed to study the effect of ewe deworming with morantel on lamb pre-weaning growth in a field experiment involving 182 lambs in 45 herds and 10 villages in Kolda, Senegal. Growth was described as a quadratic polynomial of age. Other covariates were sex, litter-size and treatment. The choice of fixed and random effects relied on three graphs: (1) a trellis display of mean live-weight vs. age, to select main effects and interactions (fixed effects); (2) a trellis display of individual growth curves, to decide which growth-curve terms should be included as random effects and (3) a scatter plot of parameters of lamb-specific regressions (live-weight vs. quadratic polynomial of age) to choose the random-effects covariance structure.

Age, litter-size, age×litter-size, litter-size x treatment and age xlitter-size xtreatment were selected graphically as fixed effects and were significant (p<0.05) in subsequent statistical models. The selection of random-effect structures was guided by graphical assessment and

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comparison of the Akaike's information criterion for different models. The final random-effects selected included no random effect at the village level but intercept, age and squared-age at the herd and lamb levels. The structure of the random-effects variance-covariance matrices were blocked-diagonal at the herd level and unstructured at the lamb level. An order-1 autoregressive structure was retained to account for serial correlations of residuals. Smaller residual variance at 90 days than at younger ages was modeled witb a dummy variable taking a value of 1 at 90 days and 0 elsewhere.

Ewe-deworming with morantel during the rainy season lead to higber latnb live-weights (probably related to a better ewe-nutrition and -health status). A positive correlation was demonstrated between early weight and growth rate at the population level (with important lamb and herd-level random deviations). The persistence of this correlation at older ages should be checked to determine whether early weigbts are good predictors of mature weights and ewe-reproductive lifetime performance.

Keywords: Linear-multilevel growth model; Random effect; Graphical methods; Pre-weaning growth; Gastro-intestinal parasitism; Morantel; Sheep; Senegal

1. Introduction

Studies in veterinary medicine frequently measure phenomena repeated over time (such as live-weights of the same animals). The analysis of these measures as multivariate outcomes over time is usually more informative than repeated cross-sectjonal studies, e.g. live-weights at given ages or cumulated production data such as 305-day milk yield for dairy cows (Gröhn et al., 1999). In addition, veterinary studies often rely on multilevel designs, in which a number of units at different levels (for example: villages, herds within villages, and animals within herds) are sampled. Data arising from these studies often have both longitudinal and multilevel features. Mixed-effect models have been used to analyze such longitudinal and multilevel data appropriately (Diggle et al., 1994). They provide a choice of effect type (either fixed or random) depending on study design and objectives at different grouping levels. The statistical properties of linear-multilevel models have been well described in recent years (e.g. Goldstein, 1986, 1995'; Pinheiro and Bates, 1996) and different software developed to fit them (Kreft et al., 1994; Littel et al., 1996).

Linear-multilevel growth models have been applied recently in veterinary studies (e.g. Puyalto et al., 1997; Green et al., 1998). However, because of their complexity and the different inferences that can result from different constructs of fixed and random effects (McDermott et al., 1997), care must be taken in mode1 specification. Model-specification issues include the choice of fixed and random effects at different levels and the structure of random-effect variance-covariance matrices. The recent availability of software combining computing tools and graphjcal methods, provides new and intuitive tools for better exploring the most-appropriate mode1 structures prior to fitting and in assessing and comparing fitted models (Pinheiro and Bates, 2000).

In thjs paper, we emphasize the use of graphical methods as a tool in model building and assessment for repeated and multilevel outcomes. As an example, a linear-multilevel growth model was developed to study the effect of ewe deworming with morantel on lamb pre-weaning growth in a field experiment in several herds and villages in Kolda, Senegal. Graphical and statistical analyses focused on the following objectives: (1) to estimate the effect of morantel treatment on lamb growth correctly, (2) to derive population-averaged estimates for lamb growth curves, (3) to evaluate the importance of lamb, herd and village-level deviations around the populations values and (4) to provide information on factors influencing live-weight growth in individual lambs under field conditions. The advantages of including graphical methods in mode1 building and assessment are highlighted.

2. Materials and methods

2.1. Data

Kolda is located in the sub-humid, southem zone of Senegal. From 1983 to 1995, mean annual rainfall was 963 mm (mainly occurring between June and November). Means of maximum- and minimum-daily temperatures were 35.5 and 20.6°C, respectively. The landscape was a mosaic of forested plateaus and lowlands cultivated with rice, cotton, maize, and groundnuts. A detailed description of the farming systems in this area was given by Faugère et al. (1990). Briefly, the main ethnic group was the Fulani. Crop farming was the dominant activity but livestock production was widely practiced (cattle, sheep and goats). Sheep were mainly Djallonke (a trypanotolerant West African dwarf breed reared extensively for meat). Normally, they were left to graze freely in the bush except during the rainy season when they were tied to avoid crop damage. A high incidence of gastrointestinal helminthoses occurred in sheep during the rainy season (Tillard, 1991). An abattoir survey revealed that *Trichostrongylus colubriformis*, *Oesophagostomum columbianum*, *Haemonchus contortus* and *Strongyloides papillosus* were the most-prevalent parasites in this region (Fritsche et al., 1993).

A field experiment was designed to assess the profitability of deworming sheep flocks in this zone and was conducted from July 1987 to June 1988. Partial results were published previously (Faugère et al., 1990; Tillard, 1991). Ten villages were selected according to agro-ecosystem representativeness and accessibility criteria. Herds belonging to volunteer farmers were enrolled and all their animals ear-tagged (Faugère and Faugère, 1986). Trained surveyors recorded demographic events and growth data during fortnightly visits. All 10 villages enrolled in the study were randomly allocated to one of two treatment groups (morantel or control) using a random-number table. Five villages were allocated to the morantel group, and five villages to the control group. Forty-five herds were included in the experiment: 17 in the control villages and 28 in the morantel villages. Treatments were applied at the village level. In the morantel-treated villages, all sheep older than 3 months were drenched three times during the rainy season with an oral administration of morantel (Exhelm®, Pfizer) at 7.5 mg kg^{-1} live-weight. No sheep in the control villages and no lamb younger than 3 months in the morantel villages were given any dewormer. Thus, the treatment-effect on lambs was mediated through its **effect** on their ewe.

In Kolda, lambing distribution is bimodal, with two peaks from October to December (end of the rainy season) and March to May (end of the hot, dry season) (Clément et al., 1997). Tbese two lambing seasons define two cohorts of lambs subject to different nutritional and environmental conditions under different husbandry practices (Faugère et al., 1990), all with possible important effects on live-weight growth. Because the purpose of this paper was mainly methodological, we decided to limit the study to the cohort of lambs born from 1 October to 31 December 1987. A more-comprehensive study of the whole data set will be presented in subsequent papers.

There were 182 lambs with 922 weight measurements in this data subset. At the time of data collection (1987–1988), follow-up information was checked and stored in temporary files (Faugère and Faugère, 1993). Weights stored in these files had been adjusted for the standard ages of 15, 30, 45, 60, 75 and 90 days (raw weights were thrown away). Before analysis, these files were gathered in a relational database (Lancelot et al., 1998). Given that each lamb was weighed every 2 weeks, little information was lost on the curvature of live-weight-growth trajectories. However, because of this standardization, a few weights (<5%) were discarded. We believe this loss of information had no important effect on our results, beyond a small decrease in statistical power.

Previous studies suggested that sex and litter-size (number of lambs bom) were important factors to consider for lamb pre-weaning growth (Poivey et al., 1982; Fall et al., 1983; Tuah and Baah, 1985; Armbruster et al., 1991; Abassa et al., 1992). Thus, sex (male or female), litter-size (single or multiple: twins and triplets) and treatment (morantel or control) were the fixed-effect classification variables used.

2.2. Data analysis

2.2.1. Craphical analyses

Graphical analyses followed the three-step exploratory data-analysis (EDA) framework proposed by Cleveland (1993): (1) display the data with respect to its inherent structure and the mode1 to be fitted; (2) display the fitted mode1 and check its assumptions and (3) examine mode1 consistency with the original data. To enhance visual comparisons of different variable combinations within hierarchically structured data, Cleveland (1994) proposed the method of trellis graphics for which software was subsequently developed (Becker et al., 1996). Data are displayed on a multi-plot trellis (matrix) of graphs. A response and an explanatory variable are on the matrix axes. Conditioning explanatory variables are defined and each pane1 (cell) within the matrix shows the response vs. the primary variables for a unique combination of the conditioning variables. Some features included to enhance visual comparisons between panels are: (1) ordering graphs by numerical explanatory variables; (2) standardizing axes values across panels; (3) including grids; and (4) labeling the panels using strip labels. For this example, both mean and individual growth curves within different sex, litter-size and treatment categories were displayed graphically. This was first done for all the data and then for individual village and herd-within-village strata to assess the consistency of the mean growth rates and their variability within each explanatory variable category graphically by village and herd (e.g. visually comparing the mean growth rates of lambs by sex and litter-size class among the five treatment and five control villages).

2.2.2. Linear-multilevel growth model

The linear-multilevel growth mode1 is an extension of the mixed-effects models described by Rao (1965) for growth curves and by Laird and Ware (1982) for longitudinal-data analysis. The mode1 was specified according to the notation of Pinheiro and Bates (2000), as follows. The highest grouping level (level 3) was lamb with subscript k. The intermediate level (level 2) was the herd with subscript j. The lowest grouping level (level 1) was village with subscript i.

The mode1 for the kth lamb within the jth herd within the ith village was

$$y_{ijk} = X_{ijk}\beta + Z_{i,jk}b_i + Z_{ij,k}b_{ij} + Z_{ijk}b_{ijk} + \varepsilon_{ijk},$$
$$i = 1,\ldots,M,\ j - 1\ ,\ldots,\ M_i,\ k = 1,\ldots,\ M_{ij},\ b_i \sim N(0,\Sigma_1),$$
$$b_{ij} \sim N(0, \Sigma_2),\ b_{ijk} \sim N(0, \Sigma_3),\ e_{ijk} \sim N(0,\sigma^2 \Lambda_{ijk}), \quad (1)$$

where

- M is the number of villages: M=10 in this study.
- M_i is the number of herds in village i: $\sum_{i=1}^{M} M_i = 45$.
- M_{ij} is the number of lambs in the herd j in tbe village i: $\sum_{i=1}^{M} \sum_{j=1}^{M_i} M_{ij} = 182$.
- y_{ijk} is a vector of weights for animal k in herd j and village i, with the vector length $=n_{ijk}$ and $\sum_{i=1}^{M}\sum_{j=1}^{M_i}\sum_{k=1}^{M_{ij}} n_{ijk} = 922$ live weights. This mode1 (and related fitting algorithms) is appropriate for unbalanced data (Laird and Ware, 1982). It does not make any restriction on the number of weights per lamb (n_{ijk}), nor on the spacing between measurements. In this analysis, standardised live-weights were used because of data availability (not because of limitations in method or software).
- X_{ijk} is an $n_{ijk} \times f$ design matrix for the fixed effects, where f is the number of fixed effects.
- β is a vector of coefficients for the fixed effects, with length of $\beta = f$.
- $Z_{i,jk}$, $Z_{ij,k}$, and Z_{ijk} are design matrices for the village-level, herd-level and lamb-level random effects, respectively. Their dimensions are $n_{ijk} \times r_{\text{village}}$, $n_{ijk} \times r_{\text{herd}}$, and $n_{ijk} \times r_{\text{lamb}}$, respectively. r_{village}, r_{herd} and r_{lamb} are the number of random effects at the village, herd and lamb level, respectively.
- b_i, b_{ij} and b_{ijk} are random vectors of coefficients for the village-level, herd-level, and lamb-level random effects, respectively. Their lengths are r_{village}, r_{herd} and r_{lamb}, respectively.
- $X_{ijk}\beta + Z_{i,jk}b_i + Z_{ij,k}b_{ij} + Z_{ijk}b_{ijk}$ is the linear predictor with its fixed ($X_{ijk}\beta$) and random ($Z_{i,jk}b_i + Z_{ij,k}b_{ij} + Z_{ijk}b_{ijk}$) parts. The fixed predictor gives the population means, while the random predictor represents the deviations around these means, with village, herd and lamb components ($Z_{i,jk}b_i$, $Z_{ij,k}b_{ij}$ and $Z_{ijk}b_{ijk}$, respectively).
- Σ_1, Σ_2, Σ_3 and Λ_{ijk} are variante-covariance matrices for the village, herd and lamb-level random effects, and for residual error, respectively. These matrices are square and symmetric with ranks r_{village}, r_{herd}, r_{lamb} and n_{ijk}, respectively. For computational reasons they must be positive-definite, i.e. their eigen values must be strictly positive (Pinheiro and Bates, 2000). When needed, further constraints can be imposed to get more parsimonious matrices, such as diagonal or block-diagonal matrices. Each term on the diagonal (variance) or off-diagonal (covariance) is a random parameter,

estimated by a complex, computationally intensive and iterative process. An important role of graphical analysis is to guide the specification of an explanatory but parsimonious random-effects structure. If residual autocorrelation exists, it can be parameterized by Λ_{ijk}, containing a set of parameters ρ (Diggle et al., 1994). Again, fitting algorithms are slow and subject to convergence problems, because a strong numerical competition occurs during the fitting process between Σ- and /I-matrices parameters (Davidian and Giltinan, 1995). Graphical analysis can play a key role in highlighting whether both autocorrelation and/or covariance parameters are required and in what mode1 form.

- σ^2 is the vector of residual variance. Additional parameters, η, to allow for non-constant variance over the growth period can be considered. Again, as for the estimation of Σ- and /i-matrices described above, graphical analysis can be very valuable.

Models were fitted using the restricted maximum-likelihood (REML) method (Patterson and Thompson, 1971). Models with identical fixed effects — but different random effects — were compared according to the Akaike's Information's Criterion (Akaike, 1974), where AIC=-2 x log-likelihood+2 x npar, with npar being the number of fixed and random parameters in the model. Mode1 likelibood (which is always higher when adding new parameters) is penalized by the number of parameters estimated. AIC is thus a trade-off between maximizing likelihood and minimizing the number of parameters to be estimated. The AIC is increasingly used in regression analysis (e.g. Venables and Ripley, 1999, pp. 185-188). Using this formulation of the AIC, the mode1 with the smallest AIC was retained.

2.2.3. Modeling strategy

Growth curves were fitted by polynomials of age, which is a common practice in growth analysis (e.g. Rao (1965) or Lee (1991) for theory, van der Leeden et al. (1996) for a comparison of methods and software, and Green et al. (1998), Orji and Steinbach (1981) for applications). Fixed effects (main effects and interactions) were chosen according to a trellis display of live-weight means against age, given sex, litter-size and treatment. A visual inspection of the shape of the average-growth curves determined the order for the age polynomial. Main effects and interactions were selected based on *visible* between-group differences in growth-curve intercepts and slopes. Particular attention was paid to assess differences in slopes (growth-rate differences) among the different panels, revealing two-way (and possibly higher-order) interactions with age. These graphically chosen fixed effects were retained in subsequent random-structure selection steps.

Trellis displays were also used to assess village-, herd- and lamb-level random effects. As a first step, mean growth curves for each sex and litter-size category were compared between treatment and control villages and herds within villages for consistency of effect. Individual-live-weight trajectories were then plotted for each combination of sex, litter-size and treatment for all the data and then for individual village and herd-within-village strata. Variations in growth-curve shapes, intercepts and slopes were examined to select possible random effects.

A number of methods were used to assess patterns of correlation graphically over the growing period. First, ordinary least-squares (OLS) regressions were performed on each lamb; live-weight was the response and a polynomial of age (plus the intercept) was the explanatory variable. Thus, $q+1$ parameters were estimated for each lamb, where q was the order of the polynomial (e.g. three parameters for a quadratic polynomial Of age: the intercept and linear and quadratic parameters). Scatter plots of the OLS parameters were used to assess the variability of each parameter and the between-parameter correlation. Plots for each village and herd were also constructed and compared. These indications were used to specify the random-effects covariance structure for the matrix Σ_3 and whether it might vary for different villages and herds.

Second, plots of the empirical autocorrelation function (Diggle, 1990, pp. 34–44) were used to check the serial correlation of the residuals. Most likely correlation structures (matrix Λ_{ijk} the residual variance) were chosen for further investigation according to these plots.

Third, the need for an even more complex residual variance structure (allowing for variance terms to vary over the growth period) was assessed using plots of residuals vs. fitted values and residuals against explanatory variables.

The results of these graphical comparisons and the number of observations at each level helped to define the strategy for comparing specific random-effects structures at different levels. Because most observations were available at the lamb level, more-complex random-effect structures suggested as necessary by graphical analyses could be fitted initially and then subsequently assessed for possible simplification. For village- and herd-level random-effect structures, fewer observations were available. Thus, parameters that were considered to be important based on graphical analyses were tested by adding them to null models including common village and herd correlation terms. In each case, the AIC was used to compare different random-effect structures.

Once the random-effect structure was established, the significance of the fixed effects was assessed (with F tests) and approximate confidence intervals parameters were obtained (using a normal approximation to the distribution of the REML estimators) (Pinheiro and Bates, 2000). Plots of observed vs. fitted values were drawn to check the goodness of fit. Normal distributional assumptions were checked with quantile–quantile plots (Cleveland, 1993).

3. Results

3.1. Model structure

3.1.1. Selection of fixed effects for subsequent modeling

The number of lambs in each sex, litter-size and treatment category are listed in Table 1. Sample-mean live-weight growth curves (for each combination of the explanatory variables: age, sex, litter-size and treatment) were plotted as a trellis display in Fig. 1. Mean curves had simple shapes that were well fitted by linear or quadratic (mostly for lambs bom in single litters) polynomials. A quadratic polynomial was retained to mode1 the age-live-weight relationship in subsequent regression analysis. Sex of lambs was not associated with growth, in any litter-size or treatment category. A litter-size effect was observed in each treatment group, both for intercepts (main effect) and

Table 1
Number of lambs in each of the main cross-classification strata (182 lambs bom in 45 herds and 10 villages in Kolda — Senegal, between October and December, 1987)

Sex	Litter-size[a]	Treatment	Number of lambs
Female	Single	Control	12
		Morantel	29
	Multiple	Control	18
		Morantel	26
Male	Single	Control	22
		Morantel	41
	Multiple	Control	1.5
		Morantel	19

[a] Sixty-eight twin and 10 triplet lambs were combined in the "multiple" category.

slopes (agexlitter-size interaction). For single lambs, mean growth curves were similar for lambs in the control and morantel groups. However, for multiple lambs, the slope was higher for the morantel than for the control groups. Because the intercepts (live-weight at 15-day of age) were close and the slopes differed, there was evidence for an agex litter-sizextreatment interaction. We decided to retain age, litter-size and treatment main effects and age xlitter-size, litter-size xtreatment and age xlitter-size xtreatment

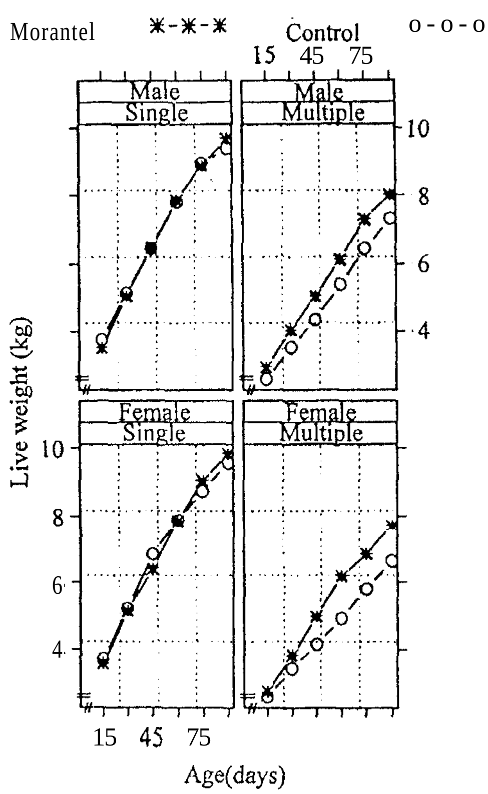

Fig. 1. Mean live-weight growth curves for 182 Djallonke lambs born in 10 villages in Kolda (Senegal) between October and December, 1987. The strip labels at the top of each panel indicate the sex (male of female) and the litter-size (single or multiple) categories.

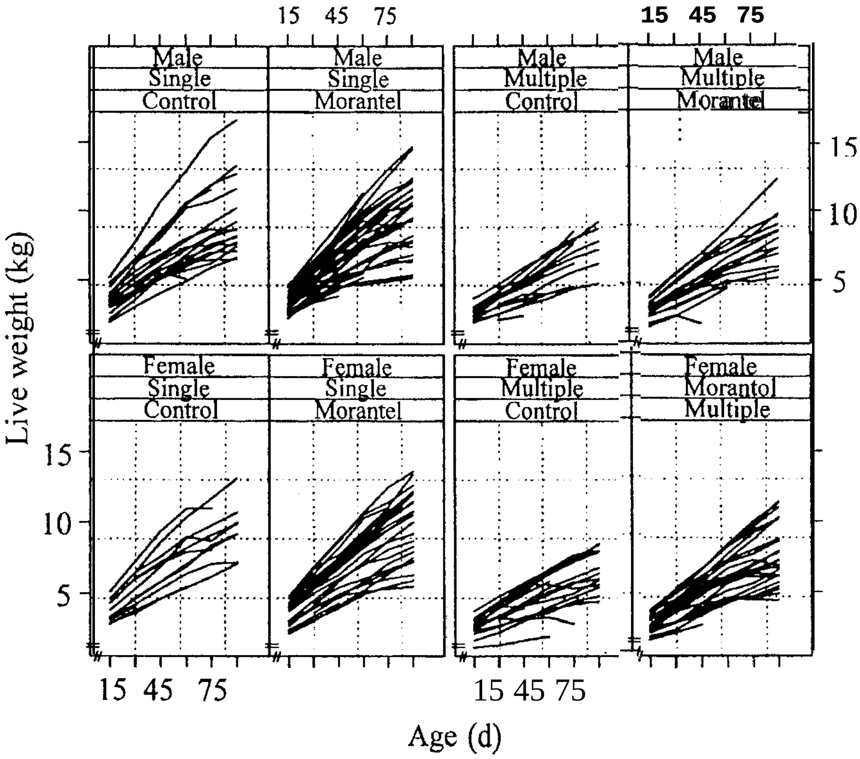

Fig. 2. Individual live-weight gmwth curves for 182 Djallonke lambs bom in 10 villages in Kolda (Senegal) bctween October and December, 1987. The strip labels at the top of each panel indicate the sex (male of female), the litter-size (single or multiple) and the treatment (contml or Morantel) categories.

interactions as the fixed effects. The agextreatment interaction was also included because of the presence of the second-order interaction (agexlitter-sizextreatment).

3.1.2. Selection of random effects for subsequent modeling

Individual growth curves were plotted on a trellis display (Fig. 2). Most of the individual curves had simple pattems. A large variation was observed for the 15-day live-weight (indicating that a random intercept should be included). Few individual growth curves crossed each other (showing that small lambs tended to stay small and large lambs large). This observation and changes in variance with age suggested that random slopes should be included (as well as covariance parameters between intercept and slopes).

A trellis display of herd-averaged growth curves (not shown) revealed similar pattems as in Fig. 2 (supporting further investigation of herd-level random intercepts, slopes and covariance parameters). Within-village herd-averaged growth curves were plotted as a trellis display (Fig. 3). This graph shows that while patterns of growth across villages were roughly similar, most villages had very few herds. Thus, it was difficult to distinguish village from herd variability and village and herd-within-village analyses would lack power (a common difficulty in multilevel studies).

The scatter plots of OLS parameters (Fig. 4) showed large variations of the parameters on their own scale. A strong positive correlation was found between the intercept and linear age. Negative correlations were found between linear and quadratic age as well as between intercept and quadratic age. For these reasons, Σ_3 (the lamb-level random-effects

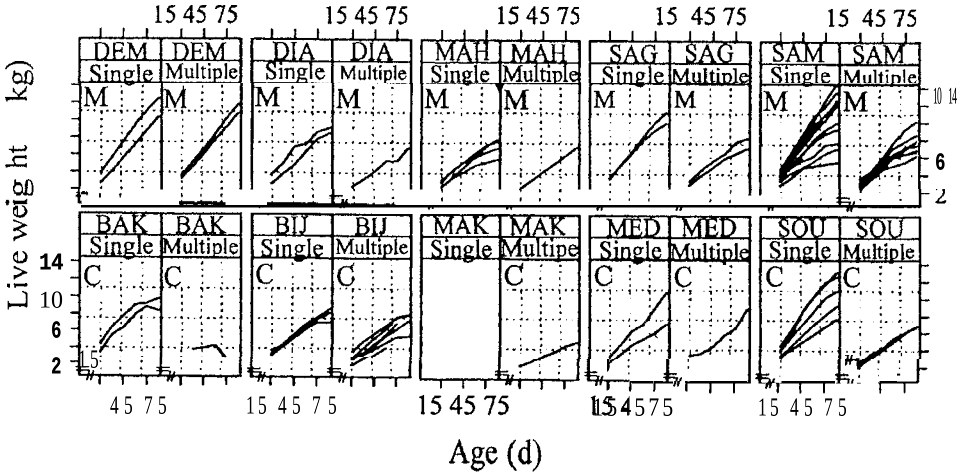

Fig. 3. Within-village, herd-averaged growth curves for 182 Djallonke lambs bom in 10 villages in Kolda (Senegal) between October and December, 1987. The strip labels at the top of each panel indicate the village (e.g. DEM) and the litter-size (single or multiple) categories. The Upper-case letter in the top, left corner of each panel stood for the treatment category: C, control; M, morantel.

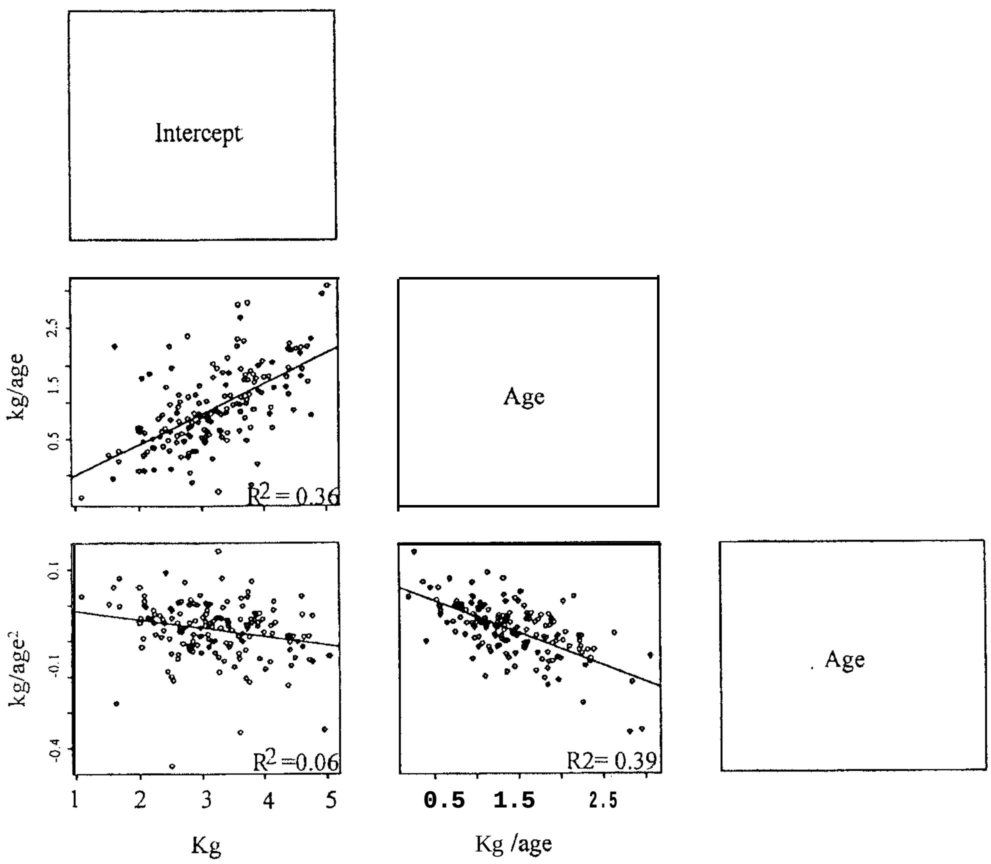

Fig. 4. Scattcrplot display of correlation between ordinary-least-squares parameters. An OLS regression was fitted for each lamb, with live-weight as the response and a quadratic polynomial for age as the explanatory variable (plus intercept). The resulting parameters (intercepf linearage and age^2) were used for the scattcrplot. The unit forage was a 15-day period. A regression line and the corresponding R^2 value were added to each plot to improve visual perception of the correlation. One large outlier (parameter for age^2=−0.6) was removed before plotting.

variance–covariance matrix) was allowed to follow an unstructured pattern (with variances for the intercept, age and squared-age on the diagonal, and covariances elsewhere (six parameters in total: three variances and three covariances)).

Because of convergence problems, we started with a rather simple random structure. An initial mode1 (fixed effects as described in Section 3.1 .1, Σ_3 as described above and random-effect intercept-only terms for village (Ci) and herd (Σ_2)) had an AIC value of 1215.26. In agreement with the graphical assessment (Fig. 3), removing the village-level random effect reduced the AIC (AIC=1213.26) very little, indicating that a village-level random effect explained very little of the variability in growth. A blocked-diagonal matrix Σ_2 was selected, with a covariance between intercept and age and no covariance between age and squared-age, nor between intercept and squared-age. This herd-level random structure provided a better fit than random-effect intercept-only, or unstructured (intercept, linear and squared-age) structures (AIC=1188.16 vs. 1213.26 and 1190.40, respectively). The lamb-level matrix Σ_3 was left unchanged.

Residuals autocorrelation was observed at lags 1 (15 days apart), 2 (30 days apart) and 4 (60 days apart) (Fig. 5, left plot). The perfect cor-relation at lag 0 is trivial but is conventionally displayed to give a better visual perception of the Y-axis scale. After trying a variety of possible serial cor-relation structures, an order-1 autoregressive [AR(l)] cor-relation structure was retained (AIC=1141.01) on the basis of AIC improvement and better autocorrelation pattern (in some cases, AIC was smaller than with AR(l), but plots of the autocorrelation function revealed disastrous cor-relation patterns). The AR(1) parameter was an estimate of α_1 in the following equation:

$$r_{ijk,t} = \alpha_1 r_{ijk,t-1} + \varepsilon_t,$$

where $r_{ijk,t}$ and $r_{ijk,\ t-1}$ were the residuals at t and t-l, respectively, and ε_t was a white-noise process with zero mean and finite variance σ_ε^2 (Diggle, 1990). Some cor-relation remained in the residuals at lag 1 (Fig. 5, right plot).

An inspection of the variation of residuals as a function of age (not presented) showed that the variance of residuals was smaller at 90 days than at younger ages. Thus, a dummy

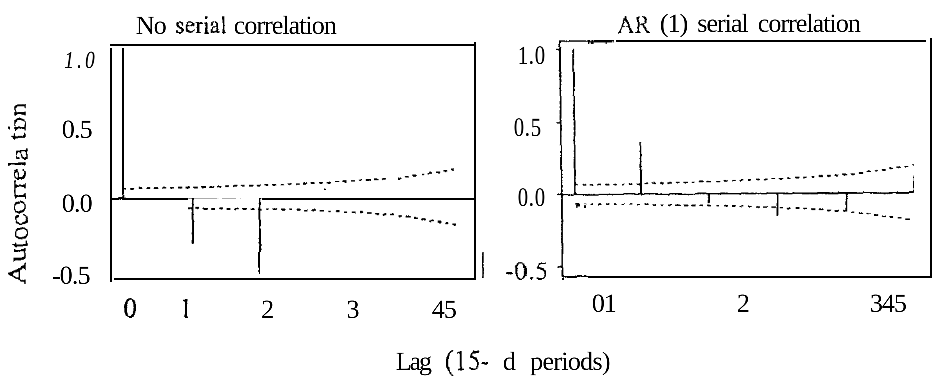

Fig. 5. Plots of the autocorrelation function for no correlation and AR(I) correlation structures for the linear-multilevel live-weight growth model of 182 Djallonke lambs bom in 10 villages in Kolda (Senegal) between October and December, 1987. The dotted lines showed the 95% confidence band for the autocorrelation. Bars outside the band indicated a significant autocorrelation for the residuals at this lag.

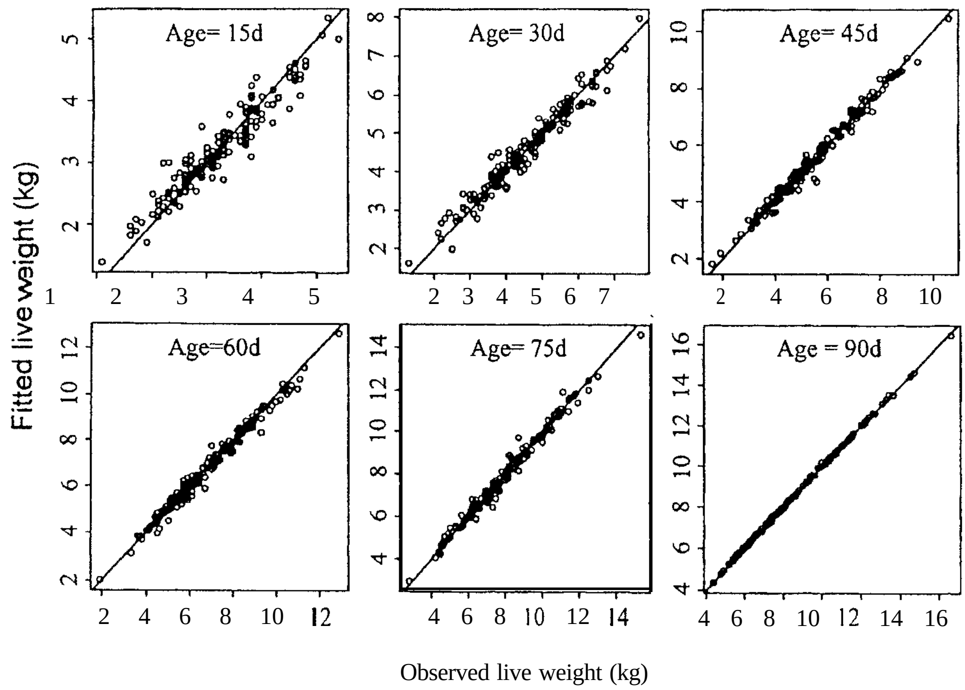

Fig. 6. Observed vs. fitted values for the linear-multilevel live-weight growth model of 182 Djallonke lambs bom in 10 villages in Kolda (Senegal) between October and December, 1987. The scales were different for each panel. Points on the bisecting line occur when the model predicts the weight observed.

variable was created, taking the values of 1 for age=90 days, and 0 elsewhere. This variable defined two strata for which separate residual standard errors were estimated. The fit of the resulting model was slightly better than a model with a single residual error (homoscedastic model) (AIC: 1140.28 vs. 1141.01, respectively).

3.2. Model diagnostics

The goodness-of-fit of the model (fitted live-weights vs. observed live-weights at each weighing age) is presented in Fig. 6. Because live-weight showed a large variation with age, the scales were allowed to vary from panel to panel, to get a better visual perception of the correlation. As expected, the fit was better at 90 days than at any other age. However, no systematic deviation from the bisecting line was observed and the overall goodness-of-fit appeared good. Quantilequantile plots (not shown) demonstrated no unusual residual pattern that might have caused us to reject the normal-distribution assumptions.

3.3. Numerical results

3.3.1. Fixed effects

Fixed-effect parameters estimates are listed in Table 2 and the population-averaged growth curves displayed in Fig. 7. A comparison of these results to those found in other West African studies for 90 days live-weight in Djallonke lambs is presented in Table 3.

Table 2
Fixed effects estimates and confidence intervals for the linear-multilevel growth model of 182 Djallonke lambs bom in 10 villages in Kolda (Senegal) between October and December, 1987

Fixed effect	Estimate	95% confidence interval[a]		p (F test)
		Lower limit	Upper limit	
Intercept	3.70	3.43	3.97	$p<10^{-3}$
Age[b]	1.64	1.45	1.83	$p<10^{-3}$
Age[2b]	-0.09	-0.12	-0.06	$p<10^{-3}$
Litter-size[c]	-1.10	-1.45	-0.76	$p<10^{-3}$
Treatment[d]	-0.13	-0.47	0.22	0.47
Age x litter-size	-0.74	-0.97	-0.51	$p<10^{-3}$
Age^2 x litter-size	0.06	0.02	0.09	$p<10^{-3}$
Age x treatment	-0.02	-0.25	0.21	0.33
Age^2 x treatment	0.03	-0.01	0.06	0.33
Litter-size X treatment	0.46	0.03	0.90	0.04
Age X litter-size X treatment	0.34	0.05	0.63	0.03
Age^2 x litter-size x treatment	-0.05	-0.09	-0.01	0.03

[a] Normal approximation to the distribution of the REML estimators.

[b] Age and age^2 were the linear and quadratic terms for age, respectively. Age was expressed in 15-day unit and the origin was set to 15 days of age.

[c] Two categories: single (reference) and multiple, the latter combining twin and triple births.

[d] Two categories: control (reference) and morantel.

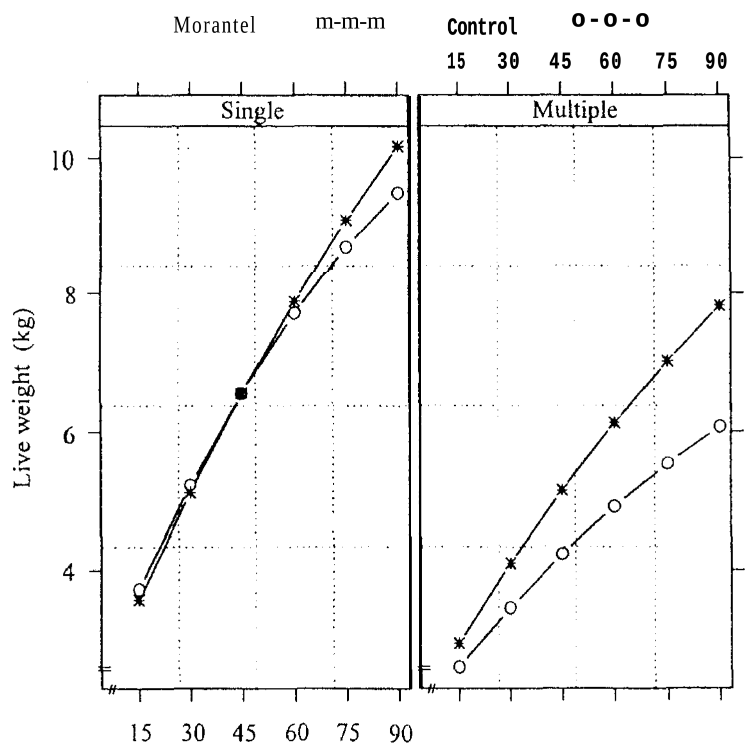

Fig. 7. Population-averagcd live-weight growth curves according to litter-size and treatment categories, prçdicted from the fixed part of the linear-multilevel live-weight growth model of 182 Djallonke lambs bom in 10 villages in Kolda (Senegal) between October and December, 1987. The strip labels at the top of each panel indicated the litter-size (single or multiple) category.

Table 3
Comparison of 90-day live-weights observed in different studies of Djallonke lambs growth in West Africa

Keference	Country	Method	Sample size	Mean	Birth type	
					Single	Multiple
This study (control lambs)	Senegal	LME[a]	182	8.2	9.6	6.3
Agyemang et al. (1991)	The Gambia	SM[b]	NA[c]	6.8	NA	NA
Armbruster et al. (1991)	Côte d'Ivoire	LME	359	8.4	9.1	7.7
Charray (1986)	Côte d'Ivoire	SM	1024	9.8	NA	NA
Fall et al. (1983)[d]	Senegal	LME	360	8.1	8.7	6.5
Poivey et al. (1982)	Côte d'Ivoire	OLS[e]	293	8.6	10.0	7.1
Sumberg and Mack (1985)	Nigeria	SM	173	8.8	NA	NA
Symoens and Hardouin (1988)	Cameroon	SM	NA	11.8	13.1	9.5
Tillard (1991)	Senegal	OLS	209	9.5	NA	NA

[a] Linear mixedeffects model.
[b] Sample mean.
[c] Not available.
[d] Spline interpolation from published results.
[e] Ordinary least-squares.

All the fixed effects considered important in graphical analyses were significant in the final mode1 (α=0.05) and those considered not important graphically (sex and its interactions with the other explanatory variables) were not significant.

A comparison of the intercepts and slopes of the growth curves of the four important treatment and litter-size categories (single vs. multiple litterxmorantel vs. control) (Table 2 and Fig. 7) showed that the higher the intercept, the higher the parameter for age, and the lower the parameter for squared-age. This latter coeffkient could be considered to be a growth deceleration parameter.

Average daily weight gains (ADWG) in grams per day were calculated from the fixed-effect estimates (Table 4). Initia1 differences in ADWG in the [15-30 days) age class were associated with parameters related to linear age (agexlitter-size, agextreatment and agexlitter-sizextreatment, see Table 2). Growth rate decreased with age. The higher the

Table 4
Average daily weight gains according to litter-size-by-treatment categories, predicted by the linear-multilevel growth mode1 of 182 Djallonke lambs bom in 10 villages in Kolda (Senegal) between October and December, 1987 (population-averdged values)

Age class (days)	Average daily weight gain (grams per day)			
	Single lambs		Multiple lambs	
	Control	Morantel	Control	Morantel
[15–30)	103	103	58	78
[30–45)	91	95	53	70
[45–60)	79	86	49	63
[60–75)	67	77	45	5.5
[75-90)	55	68	40	48

initial ADWG, the greater the decrease (i.e. growth rate decreased faster for animals with high initial weight). These different growth decelerations were associated with parameters related to squared-age (age x litter-size, age x treatment and age x litter-sizex treatment, see Table 2). However, the initial treatmentxlitter-size group live-weight rankings remained the same over all ages: single lambs had higher growth rates than multiple lambs and for multiple lambs, growth rate was higher in the morantel than in the control group.

3.3.2. Random parameters

Random parameter estimates are listed in Table 5. The correlation was $r=0.93$ between intercept weights and weights at older linear ages at the lamb level. The correlation between the intercept and square&age weights was $r=-0.60$ and between the age and squared-age weights was $r=-0.26$. A high positive correlation also was observed between the intercept and linear-age weights at the herd level ($r=0.92$).

A large overall random variation was found at the lamb-level (Table 6). The interquartile range for the random deviation from the population mean (fixed-effect prediction) was 2.6 kg at 90 days, and the range was 9.2 kg. At this age, population

Table 5
Random parameters estimates for the linear-multilevel growth model of 182 Djallonke lambs bom in 10 villages in Kolda (Senegal) between October and December, 1987

Random effect	Estimate	95% confidence interval[a]	
		Lower limit	Upper limit
Herd level			
S.D.(intercept)[b]	0.30	0.17	0.50
S.D.(age)[c]	0.21	0.13	0.34
Correlation (intercept, age)	0.92	0.23	0.99
S.D.(age^2)[c]	0.04	0.02	0.05
Lamb level			
S.D.(intercept)	0.55	0.46	0.66
S.D.(age)	0.33	0.27	0.40
S.D.(age^2)	0.03	0.02	0.04
Correlation(intercept, age)	0.93	0.45	0.99
Correlation(intercept, age^2)	-0.60	-0.87	-0.06
Correlation(age, age^2)	-0.26	-0.62	0.19
Order-1 autoregressive parameter			
a_1	0.66	0.47	0.79
Heteroscedasticity parameter			
h	0.48	0.17	1.33
Residual standard error			
s	0.34	0.26	0.44

[a] Normal approximation to the distribution of the REML estimators.
[b] Standard deviation of the random effect.
[c] Age was expressed in 15-day units and the origin was set to 15 days of age. Age and age^2 were the linear and quadratic random effects for age, respectively.

Table 6
Live-weight differences due to the rdndom effects in the linear-multilevel growth model of 182 Djallonke lambs born in 10 villages in Kolda (Senegal) between October and December, 1987

Age (days)	Random deviation (kg)					
	Herd effect		Lamb effect		Overall deviation	
	IQR[a]	Range[b]	IQR	Range	IQR	Range
15	0.3	0.9	0.7	2.5	0.9	2.9
30	0.5	1.5	1.1	4.2	1.4	4.8
45	0.7	2.2	1.5	5.8	1.9	6.7
60	0.9	2.9	1.8	7.0	2.3	7.8
7.5	1.2	3.7	2.1	7.8	2.8	9.7
90	1.5	4.7	2.6	9.2	3.3	11.6

[a] Interquartile range was the difference (due to the random effects) between the thiid and first quartiles of live-weight devidtions from the population mean.

[b] Difference (due to the random effects) between the maximum and minimum values of live-weight deviations from the population mean.

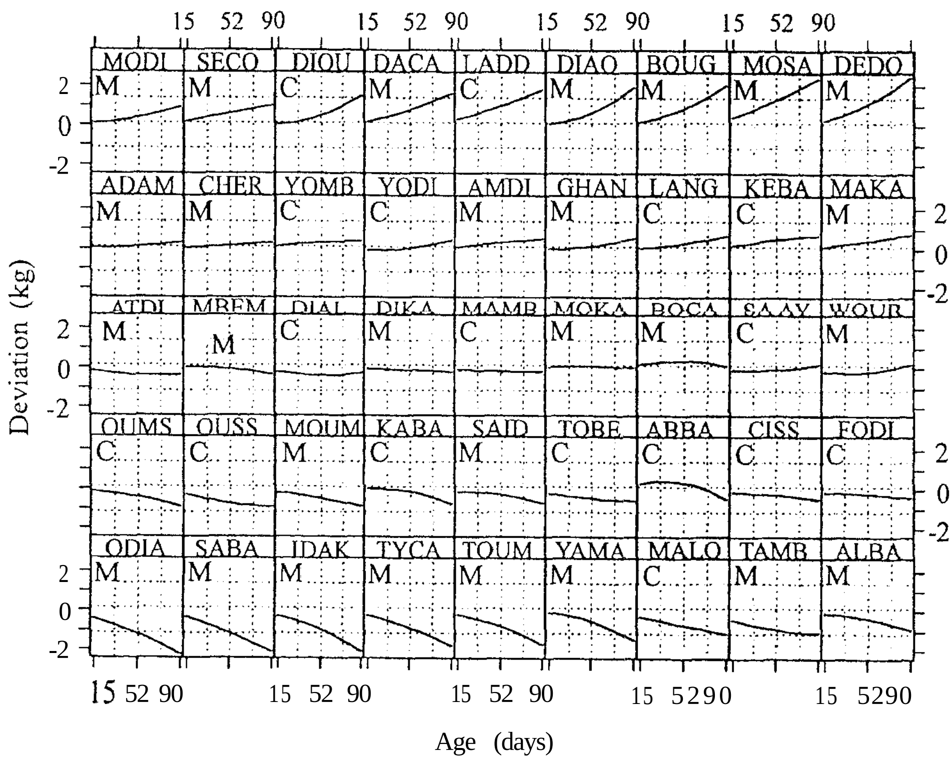

Fig. 8. Deviation from population mean due to the herd-level random effects in the linear-multilevel live-weight growth model of 182 Djallonke lambs bom in 10 villages in Kolda (Senegal) between Octobcr and December, 1987. The strip label at the top of each panel was the herd identifier. Herds were ordered from the lowest (bottom Icft panel) to the highest (top right panel) 90-day deviation. The upper-case letter in the top, left corner of each panel stood for the treatment category: C, control; M, morantel.

means, predicted from the fixed part of the model, ranged from 6.3 kg (multiple lambs, control group) to 10.0 kg (single lambs, morantel group). The herd-level variation around the population growth curve was also large (Table 6 and Fig. 8). Treatment category was not associated with the 90 days ranking of herd-level random deviations (Wilcoxon test: $W=648$, $n=28$, $m=17$, $p=0.93$).

The AR(1) parameter ($a_1=0.66$, with age expressed in 15-day periods, Table 5) indicated that residuals' autocorrelation persisted over at least 1 month (two 15-day periods). The additional heteroscedasticity parameter in Table 5 ($h=0.48$) indicated that the standard error of weight estimates at 90 days was about half as large as at other ages.

4. Discussion

The analysis of complex, multilevel, repeated-measures data requires that the structure of fixed and random-effects in the mode1 be specified appropriately for correct inferences to be made. In this example, the main study objective was to compare the growth rates of lambs of morantel-treated vs. untreated ewes. However, a correct inference of the effect of morantel treatment depended on: (1) identifying important fixed effects and their interactions that could influence the morantel-treatment effect; (2) specifying the correct random-effects structure at the multiple levels of village, herd, lamb and measurements within lamb; and (3) specifying the correct cor-relation structure between repeated weight measurements for individual lambs. This paper has demonstrated that graphical methods can play a useful role in appropriately identifying the important fixed effects and their interactions and in specifying a logical random-effect structure to guide complex model building.

In this paper, trellis graphical methods clearly and simply identified the important fixed effects and their interactions. All fixed effects highlighted by graphical analysis were statistically significant in subsequent models and those not highlighted were insignificant. This was the case for the lamb-sex effect and its interactions. Without graphical support for its exclusion, we would probably have retained it as a fixed effect with interactions, greatly increasing the rank of the fixed-effects design matrix (X) (from 12 to 24 columns). This is important for computing time, even with simpler OLS models. With larger data sets, calculations with 8-10 main effects and all their possible interactions might overwhelm present microcomputers.

Most importantly, simple graphs highlighted that a morantel-treatment effect only occurred in multiple-birth lambs. This lead to a mode1 formulation that identified a significant morantel-treatment effect that a previous analysis, using cross-sectional analysis-of-variante (ANOVA), had missed (Tillard, 1991). At the time of this first analysis, no software package was widely available to fit multilevel models. Because of the lack of power of the cross-sectional ANOVA approach, litter-size-by-treatment, and age-by-litter-size-by-treatment interactions were not found to be significant ($p>0.05$).

Graphical methods also proved very useful at identifying random-effects structures or potential problems with their estimation at multiple levels. This is crucial when analysts are confronted to new kinds of data without preliminary information on correlations between random effects. From our experience, it is not straightforward to choose an appropriate (explanatory and parsimonious) structure for the random-effects variance–covariance matrix. Cubic (or higher-order) polynomials might be necessary to model live-weight growth over a longer period of time (e.g. from birth to 1 year). The number of variance-covariance parameters in an unstructured matrix, Z, is large (10 for *each* hierarchical level with a cubic polynomial) and convergence problems are likely to occur. In such situations, graphical analysis might be the only reasonable (or even possible) way to specify a plausible and numerically stable random structure. In this analysis, initial graphical assessments were consistently confirmed in subsequent models. At the herd level, growth pattems varied and this was reflected in significant herd random intercepts and slopes. At the lamb level, trellis plots revealed more-complex correlation patterns (leading to model-building strategies starting with a complex correlation structure). For individual weight measurements within lamb, graphical methods to identify important correlations at different lags and differences in residual variation at different ages also provided useful information. In our opinion, the graphical analyses used were a powerful tool in guiding mode1 building and in effectively displaying important results.

Dam might have been considered as a grouping level in this analysis. As a matter of fact, lambs bom from multiple litters might share common and unobserved genetic, nutritional and health features, inducing cor-relation among measurements from the same litter. This level was tested (results not shown here) but no significant random effect nor covariance parameter could be identified. This is probably because most of births were single or of small size (182 lambs from 147 ewes and 147 litters: 104 single, 68 twin and 10 triple lambs) and/or the litter-size fixed effect accounted for within-litter correlation. With more-prolific species such as West-African-Dwarf goats (for which we have similar data sets) over a longer observation period (with several litters per dam), a dam effect might be more important.

The 90-day live-weight gain results in this study were within the range of observations made in other studies in the region (Table 3). The lowest weight gains were observed in Gambia (Agyemang et al., 1991); however, few details were given of the study methods. The largest weight gains were observed in northwest Cameroon (Symoens and Hardouin, 1988) in an experimental herd under improved management (housing, supplementary feeding, herd health). Thus, their results were not likely to be representative of the usual growth performance in the local farming system. The other estimates in Table 3 were similar to those reported in this study — particularly the magnitude of the multiple birth (litter-size) effect.

The effect of litter-size on weight gain was large. Multiple lambs were 1.1 kg lighter than single lambs at 15 days of age and their growth rate was lower (regardless of age and treatment group). Low growth rate for multiple lambs indicated that their nutritional needs were not met by the ewes' milk yields. At the end of the study period, single lambs bom from morantel-treated ewes had a slightly greater growth rate than lambs bom from control ewes (Fig. 7). However, the important effect of morantel treatment was in multiple-birth lambs. At 90-day of age, multiple lambs bom from treated ewes were 1.3 kg heavier, on average, than those bom from control ewes (7.6 vs. 6.3 kg, respectively, i.e. 22% more). The morantel effect was thought to be due to a better milk yield for the dewormed **ewes** — crucial for ewes with multiple lambs (Theriez, 1984). This study took place just after the rainy season, when nutritional and health conditions were at their best with abundant forage resources and crop residues and low parasitic pressure. Morantel treatment might have an even greater effect in lambs born during the second lambing peak from March to May, when ewe nutritional stress would be greatest. Because multiple births were frequent in Kolda (43% of the lambings in this sample), the use of morantel for deworming ewes during the rainy season should have an important effect on flock productivity. A thorough assessment of the benefit of deworming should take into account its effect on other production parameters (e.g. mortality, fertility, prolificacy) as well as the cost of treatment. This was done in a companion study (Lesnoff et al., 2000). Using a seasonal population-dynamics modeling approach (steady-state model), a positive effect of deworming was found, with the financial benefit-cost ratio estimated at 3.7 [1.9, 5.4] (95% confidence interval in brackets).

However, the persistency of this positive morantel-effect on growth at older ages needs to be assessed in further field studies. Published data are available with other drugs -but to our knowledge, not with morantel for Djallonke sheep.

At the herd and lamb levels, intercept and linear-age random effects were highly correlated. The persistence of this cor-relation at older ages should be checked to determine whether early weights are as good predictors for mature weight as for 90-day weights. The major consequence would be for the selection of females. In an experiment with Scottish Blackface sheep, Gunn (1977) demonstrated that ewe mature size, sexual precocity and lifetime productivity can be affected by insufficient early growth. However, in Nigeria, an analysis of station records of Yankasa ewes showed no association between sexual precocity and litter-size, birth weight and weaning weight (Osuhor et al., 1997). However, these are not strong data (observational rather than experimental and from a specific station).

The identification of herd and lamb random effects provide important targets for specific studies to improve lamb growth. The presence of herd random effects is potentially related to differences in management, feeding and herd-health practices. Thus, studies contrasting these features in flocks with low and high growth rates should be considered. Lamb random effects might be associated with genetic differences, ewe nutritional and health status, lamb diseases and other factors at the lamb level.

Graphical methods are useful in highlighting the best- and worst-performing flocks or herds. A quick examination of Fig. 8 shows that 20% of herds (nine out of 45, bottom line) were responsible for most low weights and approximately 20% for most high weights (top line). Statistical methods that ignore these herd differences are likely to make incorrect inferences on fixed effects — particularly with respect to too small variance estimates (McDermott et ai., 1994). There are particular benefits in better specifying the fixed and random-effects structures with graphical methods. Random-effect deviations from population averages are intuitively appealing in such multilevel field studies — and if correctly specified, are more powerful at detecting differences than marginal (e.g. Zeger and Liang, 1986) or generalized-least-squares (e.g. Box et al., 1994) models (van der Leeden et al., 1996).

Another important modeling issue to which graphical tools can be applied is in the decision of how parsimonious or complex a model should be. For example, in these data, autocorrelation pattems were complex. Even an unstructured Λ_{ijk} matrix (one parameter for each lag pair, for a total of 21 correlation parameters) could not remove residual autocorrelation. Graphical assessment (Fig. 2) showed that a small proportion of lambs (less than 10%) grew irregularly and poorly and that their growth was not easily modeled by age and age-squared random effects. Deleting these poor-growing lambs to improve mode1 fit is not an option. Heroic measures to better mode1 their growth is probably not wise either. The use of a simple AR(1) mode1 to parsimoniously explain residual autocorrelation for most of the lambs, the recognition that poor-growing lambs have hard-to-mode1 growth curves (for which specific studies could be initiated if considered important) and the use of graphical methods to investigate when poor mode1 fit may influence inferences about fixed and random effects seems a sensible compromise. Structured graphical analysis with trellis graphs and other recently developed methods are important tools in efforts to effectively summarize and test biological processes with sufficient and not excessive complexity.

5. Conclusion

The graphical methods presented here are an important adjunct to the consideration of linear-multilevel growth models. Users can specify relevant starting points for the fixed and the random effects, check mode1 goodness of fit and assess distributional assumptions within the same computing and graphic environment. Until recently, this process was cumbersome and required different software packages to perform different steps (Mazumdar et al., 1999). The use of graphical methods in mode1 building and assessment should greatly enhance the ability of veterinary epidemiologists to explore different random structures and to effectively communicate their findings.

In this example, graphical analyses greatly aided in highlighting important fixed and random effects. Of particular importance was the positive effect of ewe deworming on the pre-weaning live-weight growth of multiple-birth lambs. In addition, the relationships between early weight measures and subsequent growth and the **large** variability of growth between herds were nicely demonstrated by graphical analysis of correlation patterns and herd random effects. In future, we foresee that graphical tools will become more user-friendly, allowing for interactive outputs to explore specific questions of both researchers and their clients. This should allow for more their broader application and the better integration of results from individual studies into a more general livestock systems context.

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