SETTING UP EXPERIMENTS IN VETERINARY SCIENCE: AN EXAMPLE OF VIRTUAL EXPERIMENTATION

Luc Duchateau[1], Liesbeth Lievens[2] and Paul Darius[2]
[1]Dept. of Physiology and Biometrics, Ghent University, Belgium
[2]Dept. of Statistics, Catholic University Leuven, Belgium
Luc.Duchateau@ugent.be

Experimental design and random allocation of treatments is essential in applied research to run efficient experiments from which valid conclusions can be drawn. The relevance of experimental design and randomization, however, is often not well understood by students and investigators, and therefore statisticians are only consulted for the statistical analysis. A virtual experimentation environment allows researchers to design trials in their own discipline, evaluate the consequences of their choices and recognize the importance of statistical concepts such as blocks, nesting and random assignment. The application presented here consists of a vaccination trial for the prevention of udder infection in dairy cows.

INTRODUCTION

In many applied scientific disciplines, the use of efficient experimental designs is uncommon. Additionally, treatments are often not randomly assigned to experimental units. The reason that many investigators do not appreciate the usefulness of experimental design and do not properly randomize treatments is partially due to the way in which this statistical discipline is taught, often as a rather technical course with no immediate reference to the discipline of the student. Furthermore, many experimental design courses only describe properly balanced designs (e.g. latin squares) that are often impractical.

In the remainder of this paper, we will focus on veterinary science, our own area of teaching and research. Efficient experimental designs are rarely used in this field (Festing, et. al., 2002) and animals are often not properly randomized to the treatments, or each treatment group is kept together in one location (e.g. a cage) so that the location rather than the animal becomes the experimental unit. Especially since a large part of the random variation is due to the environment (such as cage or farm) which could be accounted for by the use of appropriate experimental designs, it is important to convince scientists in this discipline of the benefits of good experimental design and the importance of randomization for the validity of their conclusions.

An alternative way to teach investigators experimental design is to confront them first with a practical scientific question, and then ask them to design an experiment, given a certain amount of resources (Shunn, et al., 1999; Darius, et. al., 2007). This approach will enable the investigator to appreciate that several different options exist and that randomization plays a key role in the different designs that can be used. After the investigator has constructed these different designs, the designs can be named, their differences, advantages and disadvantages discussed, and it can be demonstrated as well how to analyze data generated by the different designs.

In order to promote this alternative type of teaching and to present the scientific question in an environment as closely linked as possible to the real world of a veterinary scientist, a 'virtual experimentation' application was developed that enables the investigator to design vaccination trials for mastitis in dairy cattle (see hhttp://lstat.kuleuven.be/env2exp). In the application, a number of farms and cows nested in farms are available to the user to evaluate a vaccine. In the next section, this application is described in detail. Once the user has constructed a design, feedback about the usefulness of the design is given upon request, as described in the last section before the conclusions.

VIRTUAL MASTITIS EXPERIMENTATION

Vaccine development in mastitis

E. coli mastitis (udder infection with *Escherichia coli*) is one of the most important diseases in dairy cattle, especially in cows in early lactation (Burvenich, et. al., 2004). A vaccine has been developed and its effects need to be assessed in a vaccination trial before it can be

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registered. Therefore cows are randomly assigned to receive either vaccine or a placebo injection. After one month, both groups of cows are challenged with an infusion of an *E. coli* dose in the udder. Severity of the response to this challenge is evaluated by the reduction in milk production 48 hours after the infusion. The objective of a vaccination trial is to prove that the milk production reduction is less in vaccinated animals. The investigator, however, needs to take into account that there are important factors that influence both the reduction and the vaccination effect. Some factors are inherent to the animal (e.g., parity, initial milk production), but others are environmental and vary from farm to farm. Furthermore, in the study presented in this application, the effect of the challenging dose was also studied, comparing a low dose (10^4 colony forming units *E. coli*) with a high dose (10^6 colony forming units *E. coli*).

Setting up a virtual vaccination trial

In order to set up a vaccination trial for mastitis, the investigator first has to select farms and cows within these farms, and next randomly assign the treatment factors of interest to farms and/or cows. The selection of farms can be done in the region view screen (Figure 1a). A map of the region is shown with the locations of the different farms. Upon selection of a farm, its characteristics, such as the milk production and the number of cows will appear in the farm view screen (Figure 1b). Also the cows from the selected farm are shown and cows can be selected. When a cow is selected, its characteristics are shown in the cow view screen (Figure 1c). Furthermore, a selected cow can be added to the trial. In adding a cow to the trial, the investigator has to take the cow characteristics into consideration, and the conclusions that can be drawn will be related to these choices. For instance, when only uniparous cows (only one calving) are selected for participation, conclusions will be restricted to such cows.

With farms and cows selected, the next step is to decide which treatment to apply to each cow. This assignment can be done manually by the investigator. This, however, should not be done for the treatment factor under investigation. Such factor should be randomly assigned. Therefore, cows can be put together in the randomization urn (Figure 1d) and the treatment factor(s) can be randomly assigned to cows. In a similar way, a treatment factor(s) can be randomly assigned to the farm as a whole.

Running the virtual vaccination trial

Once each participating cow has been assigned to a level of vaccination and challenge, the data can be generated by the application. The reduction in milk production 48 hours after the infusion for cow *i* in farm *j*, $red_{i(j)}$, will then be generated based on the following model

$$red_{i(j)} = \mu + f_j + c_{i(j)} + \pi_{i(j)} + \delta_{i(j)} + \gamma_{i(j)} + \pi\gamma_{i(j)}$$

with μ the mean reduction, $f_j \sim \mathrm{N}(0,\sigma_{\mathrm{f}}^2)$, the random effect of farm *j*, $c_{i(j)} \sim \mathrm{N}(0,\sigma_{\mathrm{c}}^2)$, the random effect of cow *i* in farm *j*, $\pi_{i(j)}$ the fixed parity effect of cow *i* in farm *j*, $\delta_{i(j)}$ the fixed challenge effect of cow *i* in farm *j*, $\gamma_{i(j)}$ the fixed vaccine effect of cow *i* in farm *j* and $\pi\gamma_{i(j)}$ the fixed interaction between parity and vaccine effect of cow *i* in farm *j*.

The fixed effects are constants based on literature data, the random farm and cow effects are drawn from a normal distribution using the appropriate variance. The individual cow reduction parameter and the initial milk production just before infusion is then used to generate the milk production 48 hours after infusion by $milk48_{i(j)} = milk0_{i(j)} \times (1 - red_{i(j)})$. The resulting data set can then be viewed and saved.

FEEDBACK FOR THE CONSTRUCTED DESIGN

The feedback is represented according to the two different strata present, the farm stratum and the cow stratum. For each stratum, appropriate information is given with respect to the factor randomly assigned to it and its replication. If no random assignments were performed, the feedback will be simple as no proper statistical analysis can be done. A warning will be given that such data need to be handled with caution as the assignment of treatments might have happened in a subjective way. With proper randomization, feedback will be given for the two strata as follows.

The farm stratum

First, information related to the random assignment will be given. In the case that no treatment factor was randomly assigned to the farm, a message will be given that farm represents a block factor that should be added to the statistical model in order to reduce the random error variability. If a treatment factor was randomly assigned to farms, the investigator will be told that the particular treatment factor needs to be assessed relative to the farm variability.

Second, the number of participating farms will be given and critically reviewed. If there is only 1 farm selected, and no random assignment was done to the farm, it is questionable whether the obtained results can be extrapolated to other farms, and the investigator will be advised to incorporate more farms. If less than 3 farms are selected and a treatment factor has been assigned to farms, it will be reported that each level of the treatment factor has not been assigned more than once to a farm, and therefore no replicates or residual degrees of freedom are available for testing the effect of the treatment factor. If between 3 and 5 farms are selected and a treatment factor has been assigned to farm, a message will be given that there are relatively few farms in the trial, so that few degrees of freedom will be available to test for the effect of the treatment factor. It is thus unlikely that a significant effect will appear, even with a large true difference.

The cow stratum

First, again information related to the random assignment is given. In the case that no treatment factor was randomly assigned to cows, such a message will be given and it will be explained that cows within farms are merely repeated measurements and no genuine replications. The cow factor can be added to the model to estimate the sampling variance, but should not be used as error term against which to test. If a treatment factor was randomly assigned to cows, a message will be given that the particular treatment factor needs to be assessed relative to the random cow variability.

Second, the number of participating cows will be given and critically reviewed. If there are less than 5 cows to which a treatment factor was assigned, a message will be given that there are relatively few cows, so that few degrees of freedom will be available to test for the effect of the treatment factor.

CONCLUSION

The importance of using efficient experimental design and randomization is not well understood in veterinary science and therefore not often applied in practice. Too often, no conclusions can be drawn from an animal experiment because it was not set up properly.

Virtual experimentation is a teaching tool that forces investigators and students to think about the setup of experiments in their own field and to make decisions with respect to design and the random assignment of the treatments. The chosen design of the experiment, however, is not necessarily one of the balanced experimental designs found in text books, such as a split plot or a latin square. The main difference is that the student starts here from the actual practice to continue with the theory.

We have already used this application frequently for teaching. Due to the fact that the virtual experimentation application is related to their own field, students grasp the ideas behind experimental design and randomization far better. Students request to be also guided to perform the analysis of the designed trial. Such an extension is currently under development.

REFERENCES

Burvenich, C., Van Merris, V., Mehrzad, J., Diez-Fraile, A., & Duchateau, L. (2004). Severity of E. coli mastitis is mainly determined by cow factors. *Vet. Res, 34*, 521-564.

Darius, P. L., Portier, K. M., & Schrevens, E. (2007).Virtual experiments and their use in experimental design. *Int. Stat. Rev., 75*, 281-294.

Festing, M. F. W., Overend, P., Das, R. G., & Borja, M. C. (2002). *The design of animal experiments: reducing the use of animals in research through better experimental design*. Laboratory Animal Handbooks.

Schunn, C. D., & Anderson, J. R. (1999). The generality/specificity of expertise in scientific reasoning. *Cognitive Science*, *23*, 337-370.

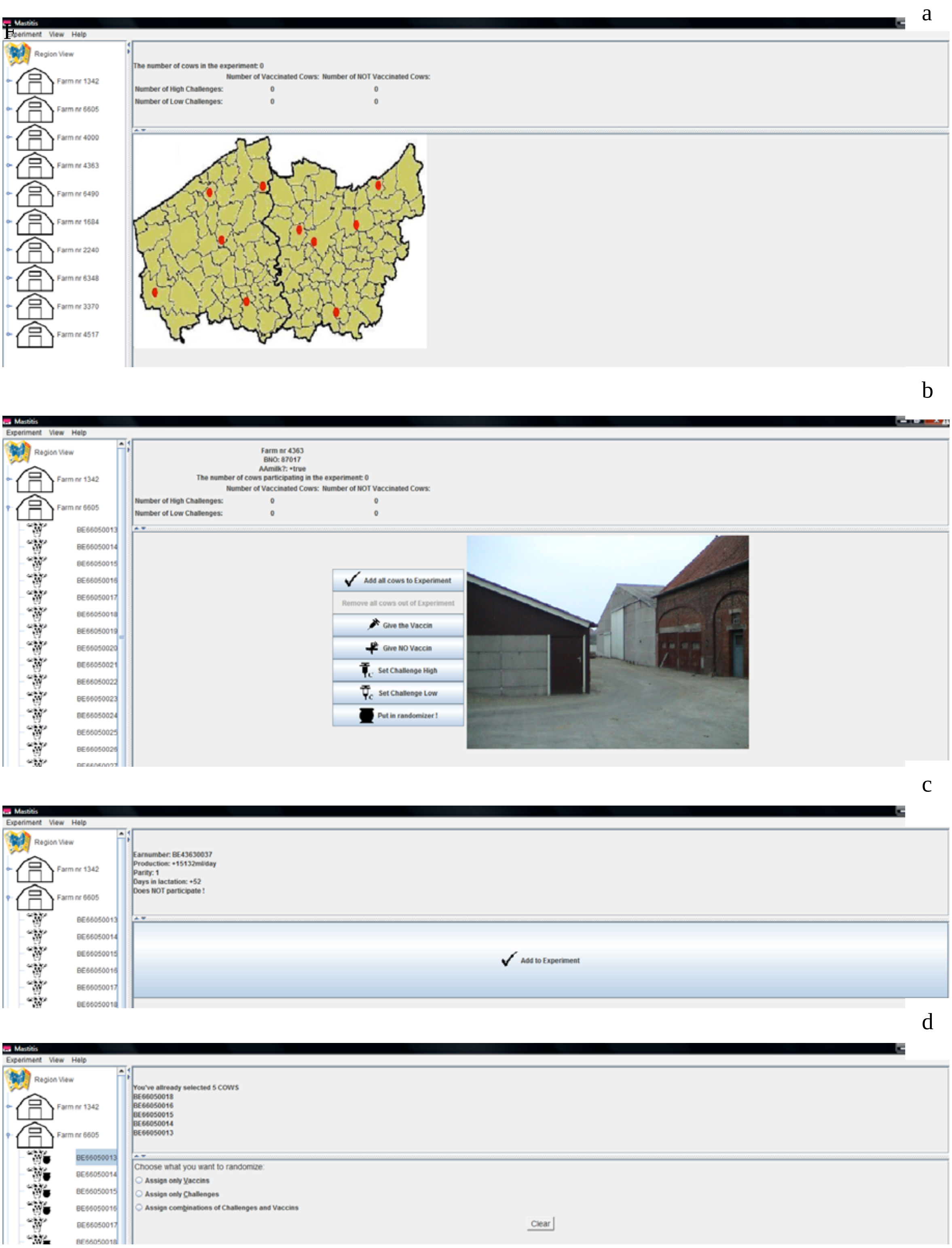

Figure 1. The virtual experimentation application for vaccination trials against mastitis with (a) the region view for farm selection, (b) the farm view for cow selection, (c) the cow view to add cow to the randomization urn and (d) the randomization urn view to randomize treatments.