Brief Description of the Survival Analysis Procedure Using the Running Rejection Behaviour of Young Rabbits as a Model Trait

István NAGY [1]
András SZABÓ [2]
Róbert ROMVÁRI [2]
Zsolt SZENDRŐ [3]

SUMMARY

The application possibilities of certain parametric and nonparametric methods concerning survival analysis were investigated on 18 Pannon White rabbits of a physiological trial. The running rejection behaviour was used as an accurate model trait for the above mentioned analysis. Rabbits had to run on a motor-driven treadmill until exhaustion twice every day. The duration of the experiment was set to be 24 days and the rabbits were arbitrarily sorted into two groups (above and below the median of the endweight of the experiment) labelled as large and small, respectively. The pattern of the results was very similar regardless of the applied method (Kaplan Meier survival curves, Cox PH model, Weibull distribution). Higher inclination towards the running rejection with the increasing weight of the rabbits seemed to be undeniable. Yet probably due to the small sample size it was impossible to obtain significant differences between the groups. Nevertheless, the appropriateness of survival analysis was clearly demonstrated in the present study and its application can strongly be recommended in order to analyse similar (i.e. time to event) data.

KEY WORDS

Running rejection behaviour, rabbit, survival analysis

[1] Department of Pig Breeding, University of Kaposvár,
7400. Kaposvár, 40 Guba Sándor Street, Hungary
E-mail: nagy@mail.atk.u-kaposvar.hu

[2] Institute of Diagnostic Imaging and Radiation Therapy, University of Kaposvár,
7400. Kaposvár, 40 Guba Sándor Street, Hungary

[3] Department of Small Livestock Sciences, University of Kaposvár,
7400. Kaposvár, 40 Guba Sándor Street, Hungary

Received: September 23, 2003

INTRODUCTION

Survival analysis is a well known and widely used statistical procedure correlating time to event data. An event might be death, developing a certain disease, or any other condition if its occurrence can be clearly detected on time scale. Applying this method it is possible to present the probability that after a certain period the event would occur during the next time unit (hazard function). It is also possible to estimate the probability that the objects of an experiment would survive longer than some specified time which means that the event has not occurred i.e. the animal is still alive, a certain disease has not yet been developed, etc. (survival function). Detailed description about the hazard [h(t)] and survival functions [S(t)] is given by Kleinbaum (1996). Survival analysis is mainly used in health sciences where the efficiency of various alternative treatments against some pre-defined illnesses can be compared with each other. Yet the method is widely used by sociologists, criminologists and also by animal scientists. The invaluable advantage of this procedure is that the so called censored data are not wasted but also used in the analysis. Censoring means that the event has not occurred till the experiment is terminated and thus the exact survival time of the participant is unknown. This phenomena either cannot be handled at all by other types of statistical analyses (like analysis of variance) or the data is reduced to being dichotomous (logistic regression) and hence considerable information is lost. By conducting the present experiment the authors had double intentions in mind. Partly, there were unanswered questions concerning the running rejection behaviour of young rabbits which were expected to be clarified through the accomplishment of the experiment. Moreover, the authors believe that the applied method is not yet well known in animal science of some countries and should deserve higher recognition and more frequent use which the present research hopes to achieve.

MATERIALS AND METHODS

The research was conducted on the experimental rabbit farm of the Kaposvar University, using 18 Pannon White rabbits. The animals were kept in a closed building in cages (800 × 500 mm), three animals in one cage and were fed ad libitum with a commercial pelletted diet (DE 10.3 MJ/kg, crude protein 17.5%, crude fat 3.6%, crude fibre 12.4%). The age of all the rabbits was four weeks at the beginning of the experiment and only male animals were used, thus the original aim of the study was to follow certain physiological changes raised by the physical load. From that cause it is reasonable to have the traits of the groups in a highly homogene manner. Thus the only differentiating factor among the animals was body weight. Though body weight was a continuous variable it was arbitrarily treated as a categorical trait. The participants of the experiment were sorted into two groups (above and below the median of the endweight) and the groups labelled as small ($S \leq 1.83$ kg) and large ($L \geq 1.84$ kg), respectively. Obviously the endweight was unknown until the end of the experiment therefore the sorting of the animals into groups could only be done at the end of the experiment which guaranteed the randomisation of the participants. The rabbits had to run on a motor-driven treadmill at a speed of 0.5-1 m/s until exhaustion twice every day. The duration of the experiment was set to be 24 days (25.10. - 18.11.2000). After a few days (survival time) some of the animals refused to run on the treadmill thus the event (or failure) occurred. On the other hand data of those rabbits that kept running till the termination of the experiment was censored (the exact period after which the rejection of the running on a treadmill would have occurred was unknown).

Sample size determination

In order to determine the appropriate sample size the method proposed by Makuch and Simon (1982) was used which defines the number of failures needed in each group. Knowing the parameters of α and β (probability of committing error type one and two, respectively) and the largest mean survival time ratio between treatment groups (a_k) the number of failures in each group (n_d) is

$$n_d = [2\tau \times (K\text{-}1, \alpha, \beta)] / [(\log_e a_k)2]$$

Given that the significance level was 5% and the power of the test was 90% ($\alpha = 0.05$ and $\beta = 0.10$) and supposing that the mean survival time ratio between treatment groups equals two (i.e. the small sized rabbits expected to run twice as long than the large rabbits) using the auxiliary table of Makuch and Simon (1982) $n_d = 44$. Supposing that some animals would show censored observation the sample size needed in the experiment is cca. 100 rabbits altogether. Unfortunately this size was far above the possibilities of the investigators. The only reasonable possibility was therefore to conduct the experiment in a sequential manner where as the data accumulates significant results can be achieved after K repetition using 2m rabbits per repetition. In order to obtain K and m respectively the table proposed by Geller and Pocock (1987) was used. In case the variance of the examined trait was $\sigma 2$ and the deviation between the large and small groups was δ moreover supposing that the ratio of $[\sigma 2/\delta] = 2.0$ was not unreasonable $K = 5$ and $m = 9$. Thus it can be concluded that using 18 rabbits per repetition dividing them into two groups (9 L and 9 S) it is necessary to conduct five repetitions of the same experiment in order to receive significant difference among the groups. Yet the authors have decided to present their preliminary results from the first repetition.

Statistical analysis

After conducting the first repetition of the experiment survival times were obtained for all the 18 rabbits (9 L and 9 S) concerning the running rejection. Analyses were carried out both with nonparametric and parametric methods respectively.

Nonparametric methods

Survival and hazard functions were obtained using the method of Kaplan and Meier (1958). Comparison of the various functions among the two groups was possible using the log-rank test. As the total running times were also recorded it was reasonable to test whether this factor should be used in order to adjust the received survival times. In order to investigate this assumption the Cox PH (proportional hazards) model was also applied (Cox, 1972). In order to check whether the Cox PH model was appropriate it was decided to use the Breslow (1974) modification of the method which plots the survival curves on a log-log scale. The application of the Cox PH model is justified when one receives parallel curves. These nonparametric tests were carried out using the SPSS software (SPSS, 1998).

Parametric methods

The main advantage of the nonparametric methods is that they can be easily applied and understood for all kinds of survival data. However in case the distribution of the survival data can truly be identified then the parametric methods should be used since they provide a more accurate description of the data. After recording the results the authors believed that from the most well known distributions which are suitable to describe the survival time properly, the Weibull distribution might be the one worth testing (Weibull, 1951). The Weibull distribution can be described by two parameters γ and λ, since

$$S(t) = e^{-(\lambda t)^{\gamma}}$$

and

$$h(t) = \lambda\gamma(\lambda t)^{\gamma-1}$$

These parameters could be estimated by the graphical method using the Weibull probability paper presented by Lee (1992a). If the two Weibull distributions (of the S and L groups respectively) do not differ from each other then $\lambda_1 = \lambda_2$ and $\gamma_1 = \gamma_2$. In order to test these hypotheses the auxiliary table was used provided by Lee (1992b) which was adopted from Thoman and Bain (1969).

RESULTS AND DISCUSSION

Though exhaustive exercise experiments are widely used in animal science and also in rabbit breeding (Frimen et al., 1986) they almost exclusively investigate the participants' metabolic and physiological responses (resulting from the exercise). As demonstrated by Meng and Pierce (1990) rabbits had the capacity to learn to run using different exercise protocols on a treadmill and therefore they could be used as appropriate model animals in the present study. However, at least to the authors' best understanding analysing the running rejection behaviour using survival analysis seems to be a unique approach. Consequently, no relevant literature was found with which to compare the received results.

Survival times and other measured parameters of the experiment participants can be viewed in Table 1.

Table 1. Basic data description

Rabbits	Group[a]	Survival time (days)	Status[b]	Endweight (kg)	Total running time (seconds)
1	S	9	1	1.6	2825
2	S	14	1	1.61	3645
3	L	9	1	1.84	3071
4	L	6	1	1.92	2945
5	S	5	1	1.78	2950
6	L	5	1	1.98	2880
7	S	5	1	1.82	2460
8	L	14	1	1.84	3330
9	S	14	1	1.74	2810
10	L	14	1	2.15	3330
11	S	24	0	1.71	3410
12	S	24	0	1.83	3705
13	L	5	1	2.09	1915
14	L	5	1	1.98	2110
15	S	10	1	1.73	2975
16	S	11	1	1.75	2955
17	L	24	0	1.87	1720
18	L	8	1	1.89	345

[a]Group S, L, endweight (S) $\leq$ 1.83 kg, (L) $\geq$ 1.84 kg; [b]Status 0, 1, censored, failed.

Nonparametric methods

The results of the Kaplan-Meier method are presented in Figure 1.

From Figure 1 it can be clearly seen that the survival curve of the S group (endweight below the median) was higher than that of the L group (endweight above the median). Nevertheless it could not be concluded that there was any difference among the groups because the log-rank test showed the significance as 0.39. Analogously the hazard function of the L group (see Figure 2) increased more rapidly than that of the S group.

Though the results presented thus far were not significant (as it was expected from the sample size determination) their tendency showed that the larger rabbits tend to reject the idea of running on a treadmill sooner than it was the case with smaller ones. Provided that the difference is not time dependent using the Cox PH model (Cox, 1972) it is possible to present a hazard ratio among the groups. After conducting such analysis a hazard ratio of 2.637 was found. Though again the result was highly insignificant (p=0.7) its meaning - i.e. the chance of the rabbits denying the running activity at any time was 2.637 times greater in the large group than in the small group - was not at all unreasonable. Checking the validity of the Cox PH model is given in Figure 3.

It can be seen that though the application of the method cannot be fully justified the survival curves are at least roughly parallel which makes the interpretation of the results meaningful.

Parametric methods

Conducting the graphical method using the Weibull probability paper presented by Lee (1992a) it was possible to determine the needed parameters: $\lambda_1 = 0.074$, $\gamma_1 = 3.9$; $\lambda_2 = 0.08$, $\gamma_2 = 2.2$. It has to be mentioned that though the data did not fit perfectly on a straight line nevertheless the deviation seemed to be in the acceptable range. The pattern of the estimated survival times (Figure 4, 5) was similar to those obtained thus far, namely the survival curve of the small group was higher than that of the large group. Moreover the received survival curves were considerably higher compared to those obtained through the Kaplan-Meier method. It is well probable that the Weibull distribution was suitable for describing the available data except for the latter period of the experiment where a clear alteration was found between the results presented by the Weibull distribution and by the other methods. However, possibly due to the small sample size the null hypothesis of $\lambda_1 = \lambda_2$ and $\gamma_1 = \gamma_2$ could not be rejected (i.e. the two Weibull distributions did not differ from each other significantly).

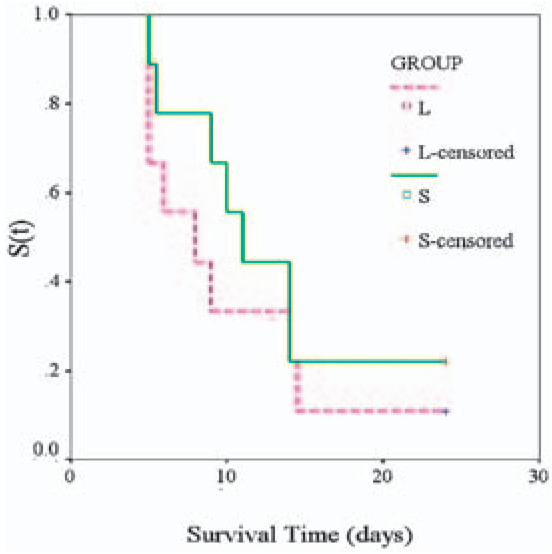

Figure 1. Survival curve of the L and S groups

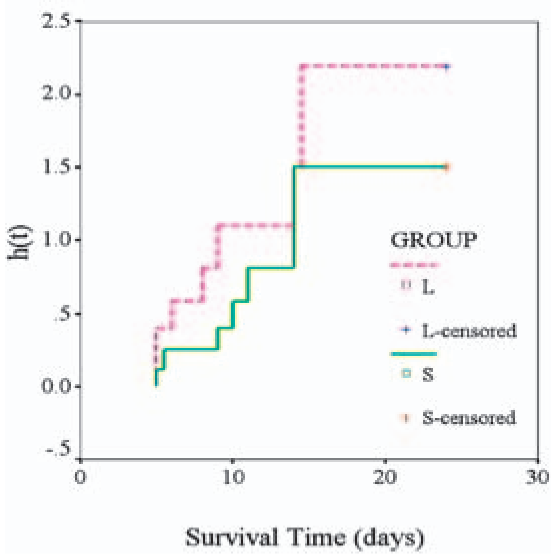

Figure 2. Hazard function of the L and S groups

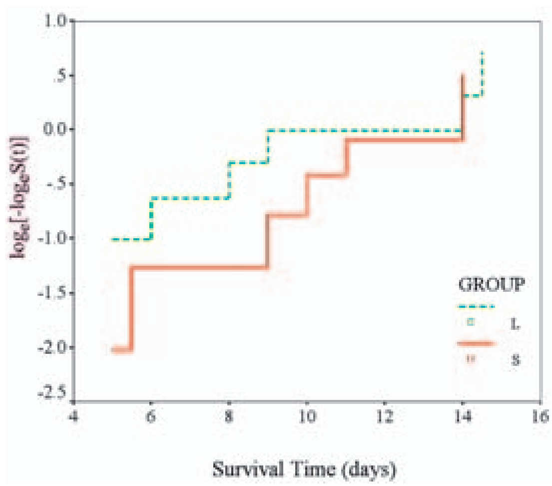

Figure 3. The validity check of the Cox PH model

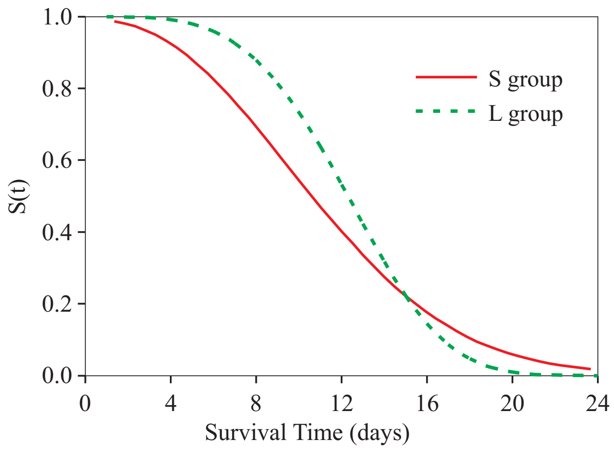

Figure 4. Survival times of the S and L groups (Parametric method: Weibull distribution)

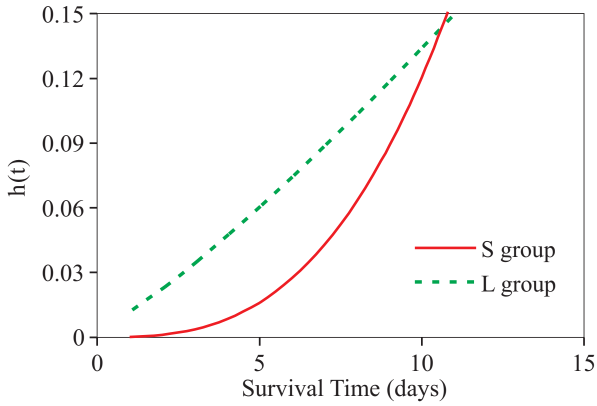

Figure 5. Hazard function of the S and L groups (Parametric method: Weibull distribution)

CONCLUSIONS

Based on the present study it could be concluded that the survival analysis was highly appropriate in order to investigate the available (time to event) data, therefore its use can be highly recommended. Due to the small sample size no significant differences were found among the two groups though higher inclination towards the running rejection (at any time during the experiment) is very likely with the increasing endweight of the rabbits. Whether the applied parametric method (Weibull distribution) is more appropriate than the nonparametric methods (Kaplan Meier, Cox PH, Breslow methods respectively) remains to be seen once the authors will have obtained accumulated data from the future repetitions of this experiment.

REFERENCES

Breslow, N. (1974): Covariance Analysis of Survival Data under the Proportional Hazards Model. Int. Stat. Rev. 43, 43-54.

Cox, D. R. (1972): Regression Models and Life Tables. J. Roy. Stat. Soc. 34: 187-220.

Friman, C.; Eronen, I.; Videman, T. (1986): Effects of Treadmill Running on Plasma Glycosaminoglycans in Adult Rabbits. Int. J. Sports Med. 7, 330-332.

Geller, N. L.; Pocock, S. J. (1987): Interim Analyses in Randomized Clinical Trials: Ramifications and Guidlines for Practitioners. Biometrics. 43, 213-223.

Kaplan, E. L., Meier, P. (1958): Nonparametric Estimation from Incomplete Observations. J. Am. Stat. Assoc. 53, 457-481.

Kleinbaum, D. G. (1996): Survival Analysis. Springer-Verlag, New York, pp. 9-14.

Lee, E. T. (1992a): Statistical Methods for Survival Data Analysis. John Wiley & Sons, Inc., New York, p. 167.

Lee, E. T. (1992b): Statistical Methods for Survival Data Analysis. John Wiley & Sons, Inc., New York, p. 450.

Makuch, R. W.; Simon, R. M. (1982): Sample Size Requirements for Comparing Time-to-Failure among k Treatment Groups. J. Chronic Dis. 35, 861-867.

Meng, H.; Pierce, G. N. (1990): Metabolic and physiological response of the rabbit to continuous and intermittent treadmill exercise. Can. J. Physiol. Pharmacol. 8, 856-862.

SPSS, (1998): SPSS-S User's Guide. 3rd ed. Chicago.

Thoman, D. R.; Bain, L. J. (1969): Two Sample Tests in the Weibull Distribution. Technometrics. 11, 805-815.

Weibull, W. (1951): A Statistical Distribution of Wide Applicability. J. Applied Mech. 18, 293-297.

acs69_05