Brazilian Journal of Veterinary Parasitology
ISSN 1984-2961 (Electronic)
www.cbpv.org.br/rbpv
Braz. J. Vet. Parasitol., Jaboticabal, v. 28, n. 3, p. 465-472, july.-sept. 2019
Doi: https://doi.org/10.1590/S1984-29612019066

Original Article

Identification of Sindhi cows that are susceptible or resistant to *Haematobia irritans*

Identificação de vacas Sindhi que são suscetíveis ou resistentes à *Haematobia irritans*

Márcia Alves de Medeiros[1,2]; Antonio Thadeu Medeiros de Barros[3]; Franklin Riet-Correa[1,4]; Ana Rita Marques[4]; José Radmácyo Gomes Lopes[1]; Vanessa Diniz Vieira[1]; Cecilia Miraballes[4]*

[1] Hospital Veterinário, Centro de Saúde e Tecnologia Rural – CSTR, Universidade Federal de Campina Grande – UFCG, Patos, PB, Brasil

[2] Departamento de Medicina Veterinária, Centro Universitário Ritter dos Reis – UNIRRITER, Porto Alegre, RS, Brasil

[3] Centro Nacional de Pesquisa de Gado de Corte, Empresa Brasileira de Pesquisa Agropecuária – EMBRAPA, Campo Grande, MS, Brasil

[4] Plataforma de Salud Animal – PSA, Instituto Nacional de Investigación Agropecuaria – INIA, Tacuarembó, Uruguay

Received April 07, 2019
Accepted July 25, 2019

Abstract

To identify susceptible and resistant *Haematobia irritans* cows, horn flies were counted biweekly for 3 years in a herd of 25 Sindhi cows. Repeated measures linear mixed models were created including cow as a random factor. The results were analyzed by: 1) observing horn fly counts, considering fly-susceptible cows with infestations appearing in the upper quartile more than 50% of the weeks and in the lower quartile less than 20% of the weeks, and fly-resistant cows those that the number of flies was in the lower quartile more than 50% of the weeks and in the upper quartile less than 20%; 2) by the best linear unbiased predictions (BLUPs), to evaluate the cow effect on fly counts. Fly-susceptible cows were those in which the infestation appeared in the 90th percentile of the BLUPs, whereas fly-resistant cows appeared in the 10th percentile. For the observational method the individuals identified as resistant varied between 8% and 20% and 8% to 12% were susceptible. For the BLUP method, the rates of susceptible and resistant cows were 12%. The agreement among methods suggests that susceptible cows can be identified by observations of fly counts, allowing for selective breeding, culling or treatment.

Keywords: Horn fly, bovines, animal selection, integrated control.

Resumo

Para identificar vacas susceptíveis e resistentes à *Haematobia irritans*, moscas-dos-chifres foram contadas quinzenalmente durante três anos em 25 vacas de um rebanho Sindhi. Modelos lineares de medidas repetidas foram criados, analisando os resultados de duas formas: 1) pela contagem das moscas, considerando susceptíveis as vacas nas quais a infestação aparecia no quartil superior mais de 50% das semanas e no quartil inferior menos de 20% das semanas. Vacas resistentes foram consideradas aquelas nas quais o número de moscas apareceu no quartil inferior mais de 50% das semanas e no quartil superior menos de 20% das semanas; 2) pela melhor predição linear não-viesada (BLUP), para avaliar o efeito das vacas na contagem de moscas. As vacas foram consideradas susceptíveis quando apareciam no percentil 90 dos BLUPs e resistentes quando apareciam no percentil 10. O método observacional identificou 8% a 20% de indivíduos resistentes e 8% a 12% de susceptíveis. O método dos BLUPs identificou igual taxa de 12% de indivíduos susceptíveis e resistentes. A forte concordância entre estes dois métodos sugere que as vacas susceptíveis podem ser identificadas pela contagem das moscas, o que permite estabelecer seleção dos animais resistentes ou tratamento ou eliminação dos mais susceptíveis.

Palavras-chave: Mosca dos chifres, bovinos, seleção animal, controle integrado.

Introduction

Although Brazilian livestock production continues to increase, productivity remains low. Cattle parasitosis, including infestations by the horn fly (*Haematobia irritans*), is among the main factors limiting the profitability of livestock in the country (GRISI et al., 2014).

Economic losses due to horn flies have been estimated at US$ 730 million per year in the USA (FOIL & HOGSETTE, 1994) and US$ 2.56 billion per year in Brazil (GRISI et al., 2014). Additionally, control of this parasite is problematic due to the development of insecticide resistance (SPARKS et al., 1985).

***Corresponding author:** Cecilia Miraballes. Plataforma de Salud Animal – PSA, Instituto Nacional de Investigación Agropecuaria – INIA, Estación Experimental INIA Tacuarembó, Ruta 5, Km 386, 45000, Tacuarembó, Uruguay. e-mail: cmiraballes@inia.org.uy

Horn fly resistance to pyrethroids, which are widely applied in Brazil (BARROS et al., 2013; BRITO et al., 2019), increases production costs and causes environmental contamination.

Horn fly infestation of a herd is directly influenced by the proportion of susceptible animals in the herd (STEELMAN et al., 1993; JENSEN et al., 2004). Thus, the identification of individuals genetically susceptible to *H. irritans* would allow the selective treatment or removal of these individuals from the herd to reduce the overall level of herd infestation. The objective of this trial was to identify horn fly-susceptible and horn fly-resistant animals in a Sindhi herd by two different methods. The information acquired can be used in control programs to reduce insecticide use.

Materials and Methods

This trial was conducted for 42 months between April 15, 2011, and September 20, 2014, at the Federal University of Campina Grande, Patos, Brazil. No treatment against horn flies was applied during the trial.

Data collection

The number of horn flies on 25 adult cows (aged four to 10 years) from a Sindhi herd was counted every 14 days. As it was an open herd, the trial period was divided into three stages based on cow composition, with the same cows maintained within each period: 2011-2012 (36 biweekly observations); 2012-2013 (26 biweekly observations); and 2013-2014 (22 biweekly observations). Only ten cows were present in the herd throughout the entire period from 2011-2014 (84 biweekly observations).

The counts were performed by one trained observer between 7:00 am and 8:30 am. During the counts, the cows were contained in a squeeze chute. Total infestation was estimated by counting the flies on one side of each cow and multiplying by two according to the technique described by Morrison & Foil (1995) and Medeiros et al. (2018).

Statistical analysis

Three databases were created in Excel, and the data were reviewed for data entry errors. One database was created for each study period (2011-2012; 2012-2013; 2013-2014). Excel spreadsheets were imported into R version 3.5.2 (R CORE TEAM, 2013) for descriptive and statistical analysis, performed using the lme4 package (BATES et al., 2015).

Descriptive analysis

The variables evaluated were the number of horn flies on the cows, the sampling date and a binary variable for rainy or dry season. Descriptive statistics were calculated, including the median, the interquartile range, and the minimum and maximum number of horn flies, for each observation day. For the present analysis, fly-susceptible cows were identified as those for which the infestation of flies appeared in the upper quartile for more than 50% of the weeks and in the lower quartile for less than 20% of the weeks. In contrast, fly-resistant cows were defined as those for which the fly counts appeared in the lower quartile for more than 50% of the weeks and in the upper quartile for less than 20% of the weeks.

Inferential statistics

To identify resistant and susceptible cows for the best linear unbiased predictions (BLUPs) analysis, three repeated measures linear mixed models (one for each period) were constructed with cow as a random effect intercept. The response variable was the log ten transformed counts of horn flies per cow, and the explanatory variable were the observation date and season. As the trail took place in a semiarid region with two seasons well stablished (rainy and dry) the season was evaluated monthly as a binary outcome, considering a rainy season if it rained more or equal than 50mm or dry season if the rain was less than 50mm. From the previously modeled values, the BLUPs for the estimation of random effects were determined. The Standardized residuals and the BLUPs of the random effects were obtained and assessed for normality, heteroscedasticity and outlying observations as described by Dohoo et al. (2009). Each cow's BLUPs were plotted against the average quantile rank values that were determined as the difference between the number of weeks in the high-risk quartile group and the number of weeks in the low risk quartile group, averaged by the total number of weeks in each of the observation periods. A linear model fit for the values of BLUPS against the average rank values and the correlation between the two methods was tested using Spearman's correlation coefficient.

The animal effect values (BLUPs) were evaluated by percentiles, with 0 representing the lowest counts (or more resistant cows) and 10 representing the highest counts (or more susceptible cows). These BLUPs represented only the effect of cow and not the effect of day, season or other unmeasured counfounders.

Data availability

The data base is available at Miraballes et al. (2019).

Results

Based on the observational analysis, cows were identified as fly-susceptible or fly-resistant, and the results were compared with those of the BLUPs analysis (Figure 1). Results for the observational and BLUPs methods are available in Figure 2 and Figure 3 respectively. The results of the two confronted methods were largely consistent, suggesting that susceptible cows can be reliably identified by observations of fly counts. In Figure 1 it can be observed the strong linear correlation between average quartile ranks, as the averaged difference between the number of biweekly counts in the high and low quartiles, and BLUPs for each cow and for each study period. The observational method results are represented in Figure 1 as point shapes, while susceptible and resistant cows identified with the BLUPs method as encircled

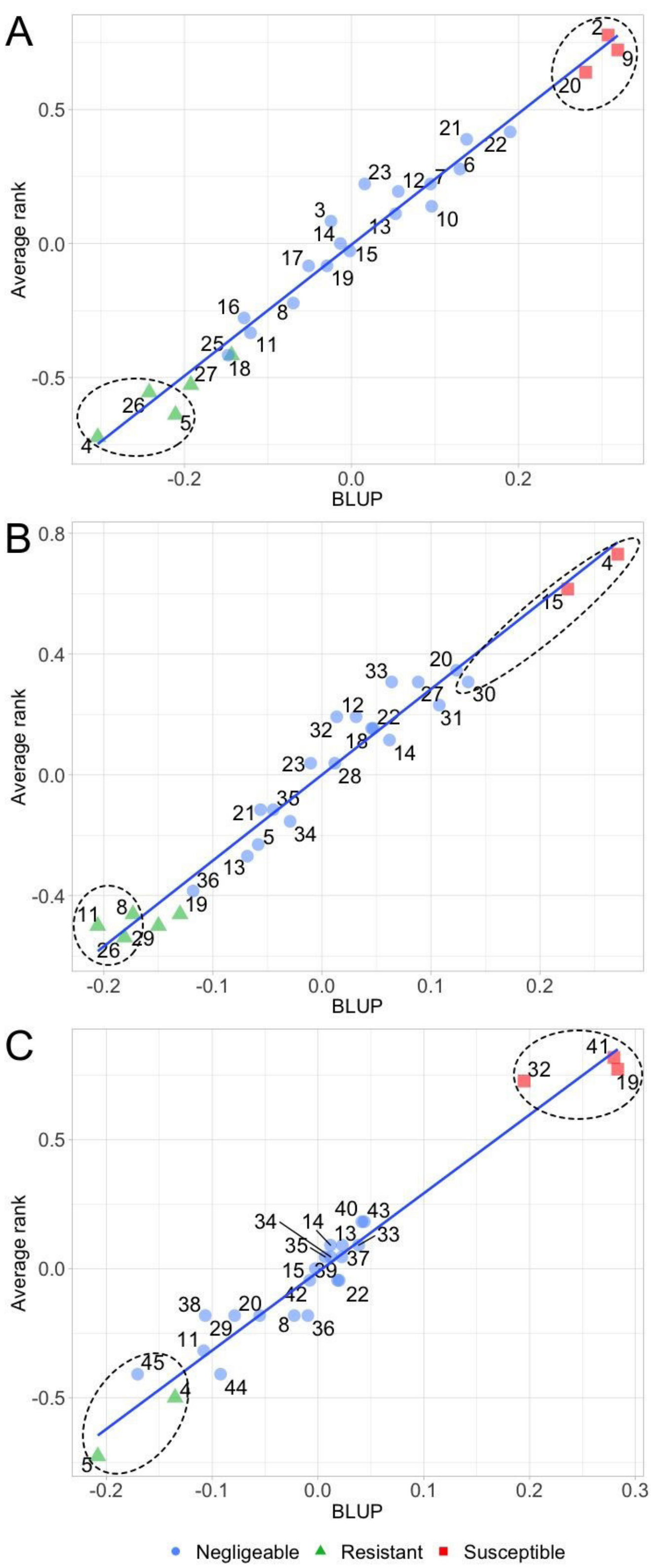

Figure 1. Average rank between the number of times that a cow was observed in the upper and the lower quartile, plotted against the BLUPs, for each study period: 2011-2012 (A); 2012-2013 (B); 2013-2014 (C). Susceptible and resistant cows are identified with encircled values for the BLUP method and with differential point shape for the observational method.

values. For the first year period, the observational method identified cow's id number 4, 5, 18, 26 and 27 as resistant (20% of the cows) and cows 2, 9 and 20 as susceptible (12%). The BLUPs method identified the same susceptible cows but fewer resistant individuals [cows id number 4, 5 and 26 (12%)]. The agreement between methods was also partial for the period of 2012-2013: two individuals were identified as resistant in the observational method (cows id number 29 and 19) that were not identified with the BLUP method, while the BLUP method identified cow id number 30 as susceptible. For the final period of 2013-2014, observational and BLUP methods differed only in classifying cow id number 45. Overall, the rates of individuals identified varied between 8% and 20% for resistant individuals and 8% to 12%

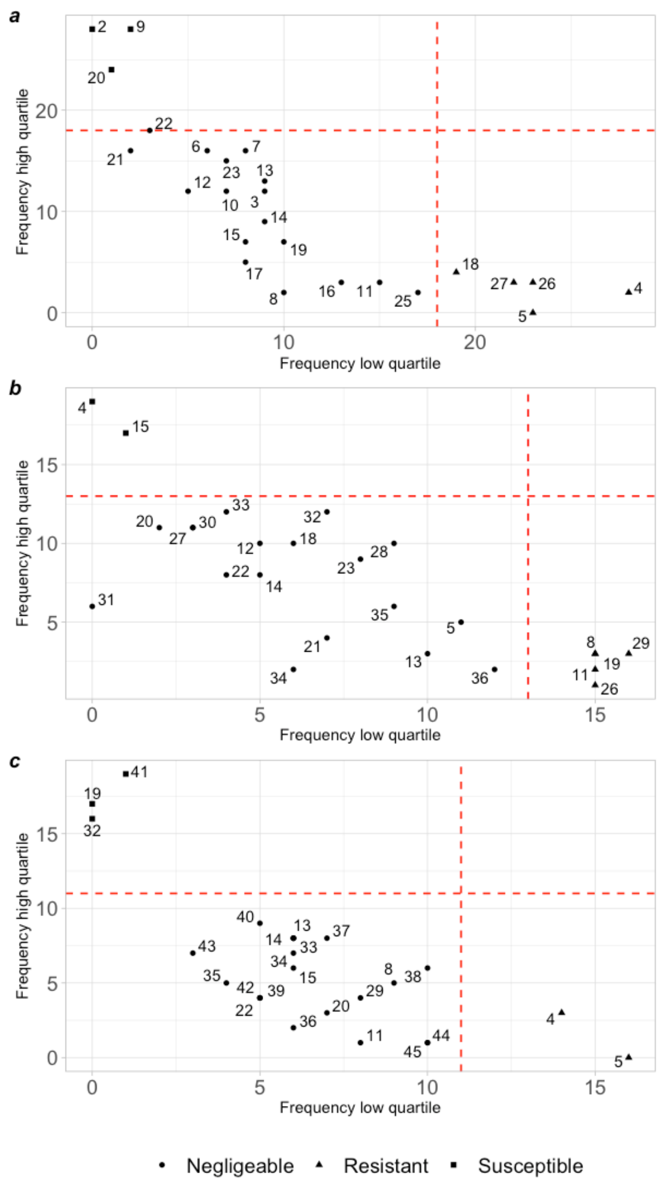

Figure 2. Quartile method plot where susceptible and resistant individuals are identified in the upper-left and lower-right plot quadrants, respectively, for 2011-2012 (a), 2012-2013 (b) and 2013-2014 (c). The red line represents 50% of the observations.

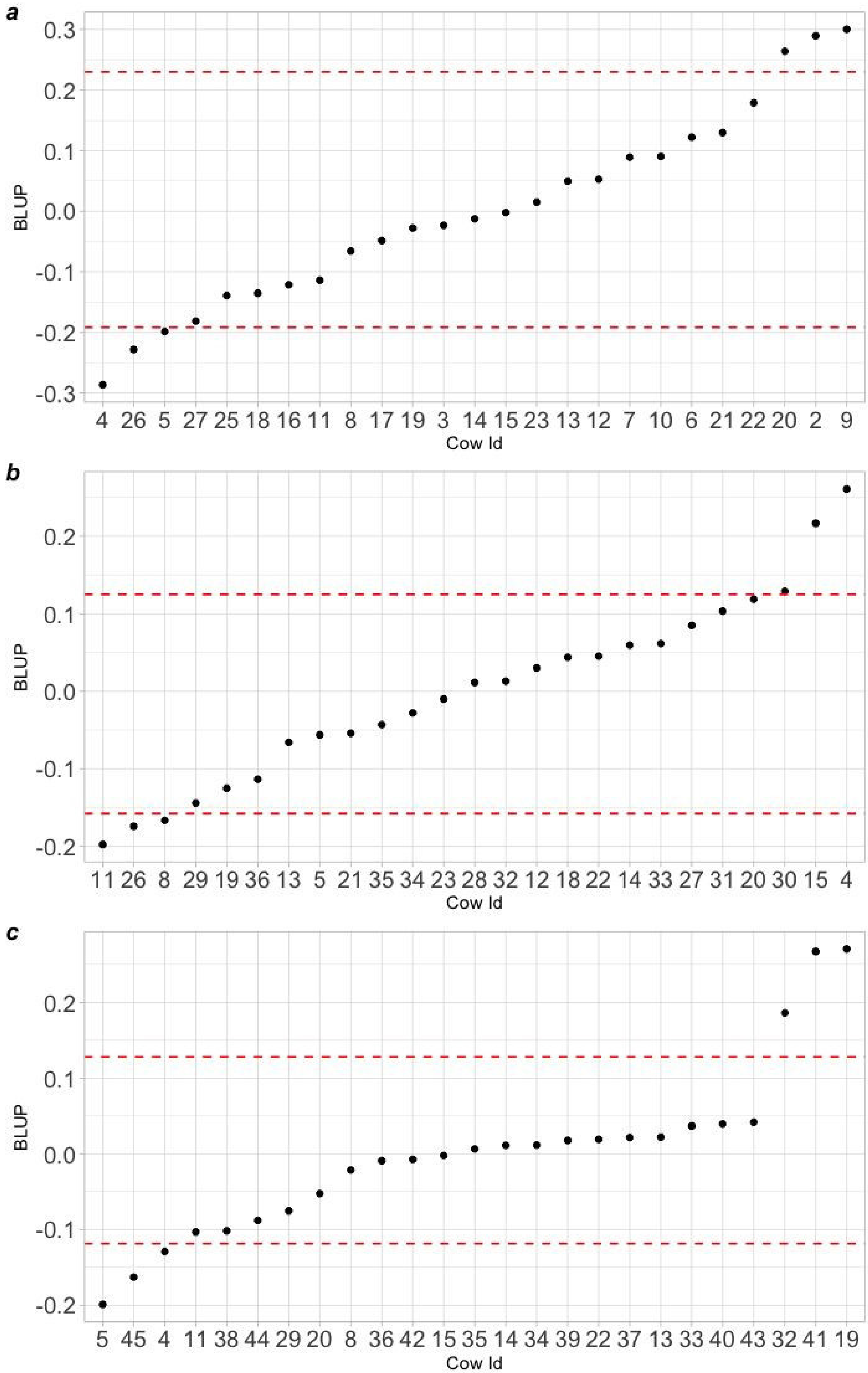

Figure 3. Susceptible and resistant cows are determined according to the value of the BLUPs of the random effects for the linear mixed model and their position in the 90th or 10th percentile, represented as the horizontal and dashed thresholds, for 2011-2012 (a), 2012-2013 (b) and 2013-2014 (c).

Table 1. Identification of fly susceptible and fly resistant cows by id according times a cow was observed in the lower or upper quartile of the weekly distribution of horn flies and to the BLUPs.

2011-2012							
Observed						BLUPs	
Fly susceptible	% of times observed		Fly resistant	% of times observed		Fly susceptible	Fly resistant
Cow id	Lower quartile	Upper quartile	Cow id	Lower quartile	Upper quartile	Cow id	
2	0.00	77.80	4	77.80	5.60	2	4
9	5.56	77.80	5	63.89	0.00	9	5
20	2.78	66.70	18	52.78	11.11	20	26
			26	63.89	8.30		
			27	61.11	8.30		
12%			20%			12%	12%
2012-2013							
Observed						BLUPs	
Fly susceptible	% of times observed		Fly resistant	% of times observed		Fly susceptible	Fly resistant
Cow id	Lower quartile	Upper quartile	Cow id	Lower quartile	Upper quartile	Cow id	
4	0.00	80.80	8	57.69	11.50	4	8
15	3.85	65.38	11	57.69	7.69	15	11
			19	57.69	11.50	30	26
			26	57.70	3.85		
			29	61.50	11.50		
8%			20%			12%	12%
2013-2014							
Observed						BLUPs	
Fly susceptible	% of times observed		Fly resistant	% of times observed		Fly susceptible	Fly resistant
Cow id	Lower quartile	Upper quartile	Cow id	Lower quartile	Upper quartile	Cow id	
19	0.00	77.30	4	63.63	13.63	19	4
32	0.00	72.70	5	72.72	0.00	32	5
41	4.54	86.40				41	45
12%			8%			12%	12%

for susceptible individuals in the observational method, while for the BLUP method, the rate of 12% was shared among years and categories (Table 1). On average, the observational method identified 16% resistant cows and 10.7% susceptible cows, while the BLUP method consistently identified 12% of the cows as susceptible and 12% as resistant. It is observed a largely consistent identification between the two methods. Furthermore, Figure 1 shows the strong linear correlation between average quartile ranks and BLUPs for each cow and for each study period. The strong correlation translates into the agreement between the two methods, with high values for the Spearman's rank correlation coefficients of 0.99 (Figure 1a), 0.97 (Figure 1b) and 0.95 (Figure 1c), and $p<0.05$).

In figure 4 are represented the number of horn flies for the three observed time periods, coded in accordance to the concurring results from the two confronted methods: for the first time period of 2011-2012 (Figure 4a), both methods identified 3 cows as susceptible and 3 cows as resistant; for 2012-2013 (Figure 4b), 3 cows were identified as resistant and 2 cows as susceptible; and for 2013-2014 (Figure 4c), 2 cows were identified as resistant, while 3 cows were identified as susceptible. Cow id number 4 was simultaneously identified by both methods as resistant for two time periods and as susceptible for the period of 2012-2013. Cow id number 19 was also classified among different groups resistant and susceptible for the two time periods using the observational method. The equivalent of Figure 4 with logged values is provided in Figure 5. In Figure 4, it can be verified how the number of horn flies exceeded the threshold for treatment (200 flies per cow) on numerous occasions, during peaks of infestation. After a peak, it is frequently observed that fly counts rapidly decreased to below this threshold, even though no treatment was administered.

The repeatability estimates were determined for each period, with 28.6% for 2011-2012; 21.5% for 2012-2013 and 24.7% for 2013-2014. The correlation among observations within the individual is consistent between time periods, reflecting the need for a repeated measures linear mixed model, and that the number of horn flies per cow is a trait, varying among cows.

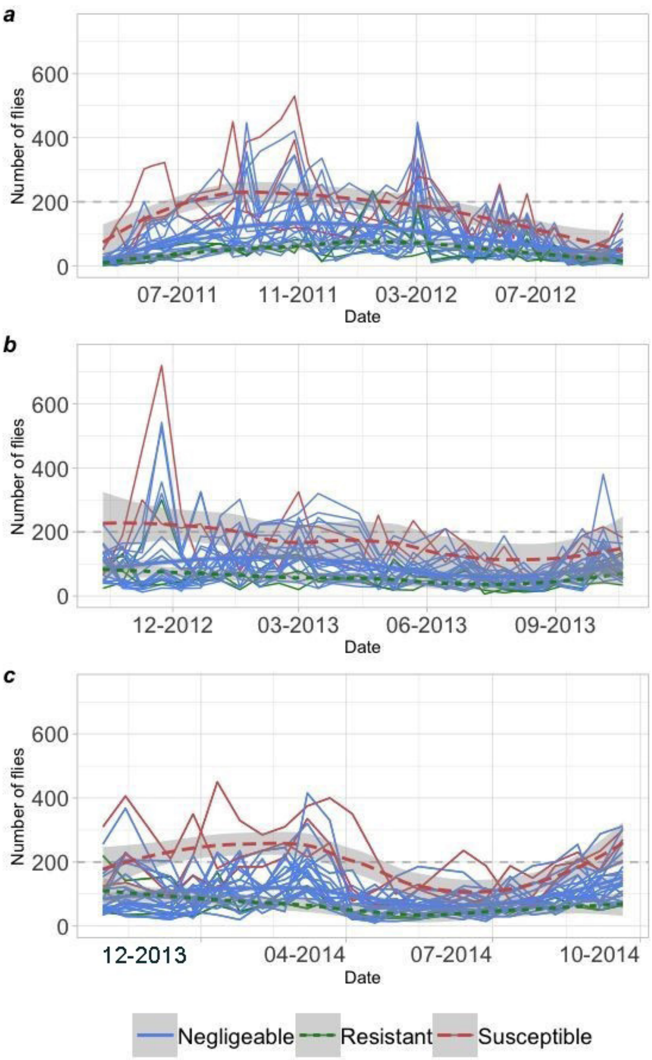

Figure 4. Observed number of horn flies on the 25 cows present throughout the three study periods, for 2011-2012 (a), 2012-2013 (b) and 2013-2014 (c). Each cow's series of values has been plotted to represent their classification for each of the three risk groups.

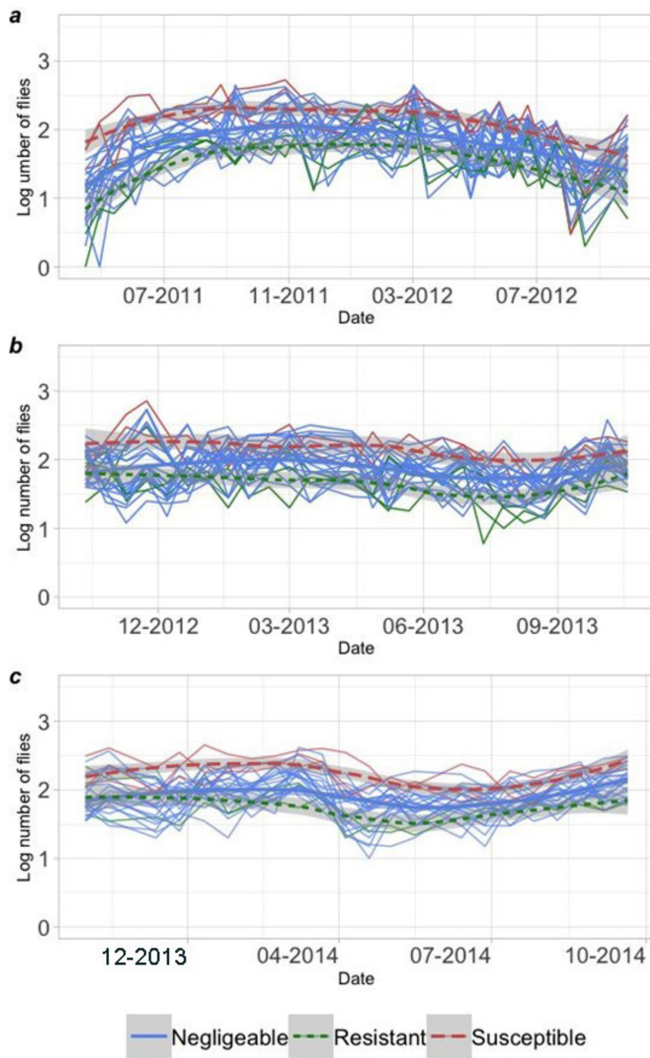

Figure 5. Observed number of horn flies' log 10 scaled on the 25 cows present throughout the three study periods of 2011-2012 (a), 2012-2013 (b) and 2013-2014 (c). Each cow's series of values has been coded to represent the classification for three risk groups.

Discussion

The mechanisms underlying individual differences in horn fly resistance or susceptibility among cows of the same breed are not well understood. Climatic and host-related factors such as breed, color, age, and behavioral defense against horn flies, among others, influence the population dynamics of horn flies (FRANKS et al., 1964; BEAN et al., 1987). In this trial, BLUPs analysis allowed the influences of these factors to be removed and the resistance or susceptibility of each animal to be evaluated, which could also be identified by observing the degree of infestation of the cows. However, one cow was considered fly resistant in two stages of the trial and fly susceptible in the third, which suggests the existence of individual variation due to unmeasured factors, such as physiological and/or immunological condition.

This methodology allows to evaluate the susceptibility of the individuals throughout the time studied. Otherwise, other reports have identified the susceptible and resistant individuals in herds at one specific day (BIANCHIN & ALVES, 1997), by counting the flies on 50% of the herd randomly selected (BARROS, 2001) or by reporting the average of the susceptible or resistant bovines in the period of study (MIRABALLES et al., 2018).

The identification of higher-susceptibility animals based on fly number allows the selective treatment of such animals, which can decrease the number of flies in the herd and the environment. Jensen et al. (2004) demonstrated that the level of infestation of a herd with *H. irritans* could be altered by adding or eliminating fly-resistant or fly-susceptible animals. Thus, it is suggested that selective treatment of the most infested animals may have the same effect as changing the composition of the herd. In a previous trial, the selective treatment of bulls decreased the rate of infestation of the untreated cows during the mating period (MIRABALLES et al., 2018). Bulls have higher infestation rates than cows; therefore, selectively treating bulls in a breeding herd would be of value. However, it is important to evaluate the implications of selective treatment for the development of insecticide resistance (BARROS et al., 2013). Since information regarding the development of resistance in selective treatment programs involving long-lasting insecticides is lacking, it is important to perform bioassays before and after treatments to evaluate the susceptibility of horn fly populations to the drugs in use.

The identification of the higher-susceptibility animals may also be valuable for genetic selection. As determined by Brown et al. (1992) and Steelman et al. (1996), the heritability of fly resistance is moderate, so selection of animals based on fly resistance, among other criteria, can be expected to reduce rates of horn fly infestation over the long term.

Barros (2001) identified frequencies of 13.3% and 15% of Nelore cows as susceptible, and Miraballes et al. (2018) found that 8.3% of cows were susceptible in a Braford herd. In both previous studies, the proportion of fly-resistant animals was greater than that of fly-susceptible ones. These findings align with those of this study. Nevertheless, it is recognized that the effect of variation in herd members could have contributed to the varying identification of cows as susceptible and resistant between study periods, as was the case of cow id number 4, similarly classified by both methods at one instance as susceptible and at two instances as resistant. Sindhi cows are a *Bos indicus* breed; thus, it is important to perform a similar study on *Bos taurus* breeds since horn fly infestations tend to be higher in European breeds (TUGWELL et al., 1969), and the proportion of susceptible animals may therefore differ between them.

Conclusion

Fly-susceptible and fly-resistant cows can be identified by observation of the number of flies on the animals, allowing the selective treatment or removal of the more susceptible individuals.

Acknowledgements

The authors acknowledge the Coordenação de Nível de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for a PhD scholarship awarded to the first author. Additionally, the authors acknowledge the employees of the Núcleo de Pesquisa para o Trópico Semiárido of Campina Grande University for assistance with animal management during the study.

References

Barros ATM, Schumaker TTS, Koller WW, Klafke GM, Albuquerque TA, Gonzalez R. Mechanisms of pyrethroid resistance in *Haematobia irritans* (Muscidae) from Mato Grosso do Sul state, Brazil. *Rev Bras Parasitol Vet* 2013; 22(1): 136-142. http://dx.doi.org/10.1590/S1984-29612013005000016. PMid:23538495.

Barros ATM. Dynamics of *Haematobia irritans irritans* (Diptera: Muscidae) infestation on Nelore cattle in the Pantanal, Brazil. *Mem Inst Oswaldo Cruz* 2001; 96(4): 445-450. http://dx.doi.org/10.1590/S0074-02762001000400002. PMid:11391414.

Bates D, Mächler M, Bolker B, Walker S. Fitting linear mixed-effects models using lme4. *J Stat Softw* 2015; 67(1): 1-48. http://dx.doi.org/10.18637/jss.v067.i01.

Bean KG, Seifert GW, MacQueen A, Doube BM. Effect of insecticide treatment for control of buffalo fly on weight gains of steers in coastal central Queensland. *Aust J Exp Agric* 1987; 27(3): 329-334. http://dx.doi.org/10.1071/EA9870329.

Bianchin I, Alves RG. *Mosca-dos-chifres: comportamento e danos em bovinos nelores*. Campo Grande: Embrapa CNPGC; 1997. Comunicado Técnico, no. 55.

Brito LG, Barbieri FS, Rocha RB, Santos APL, Silva RR, Ribeiro ES, et al. Pyrethroid and organophosphate pesticide resistance in field populations of horn fly in Brazil. *Med Vet Entomol* 2019; 33(1): 121-130. http://dx.doi.org/10.1111/mve.12330. PMid:30125976.

Brown AH Jr, Steelman CD, Johnson ZB, Rosenkrans CF Jr, Brasuell TM. Estimates of repeatability and heritability of Horn Fly resistance in beef cattle. *J Anim Sci* 1992; 70(5): 1375-1381. http://dx.doi.org/10.2527/1992.7051375x. PMid:1526906.

Dohoo I, Martin SW, Stryhn H. *Veterinary epidemiologic research*. 2nd ed. Charlottetown: VER Inc; 2009. 865 p.

Foil LD, Hogsette JA. Biology and control of tabanids, stable flies and horn flies. *Rev Sci Tech* 1994; 13(4): 1125-1158. http://dx.doi.org/10.20506/rst.13.4.821. PMid:7711307.

Franks RE, Burns EC, England NC. Color preference of the horn fly, *Haematobia irritans*, on the beef cattle. *J Econ Entomol* 1964; 57(3): 371-372. http://dx.doi.org/10.1093/jee/57.3.371.

Grisi L, Leite RC, Martins JRS, Barros ATM, Andreotti R, Cançado PHD, et al. Reassessment of the potential economic impact of cattle parasites in Brazil. *Rev Bras Parasitol Vet* 2014; 23(2): 150-156. http://dx.doi.org/10.1590/S1984-29612014042. PMid:25054492.

Jensen KMV, Jespersen JB, Birkett MA, Pickett JA, Thomas G, Wadhams LJ, et al. Variation in the load of the horn fly, *Haematobia irritans*, in cattle herds is determined by the presence or absence of individual heifers. *Med Vet Entomol* 2004; 18(3): 275-280. http://dx.doi.org/10.1111/j.0269-283X.2004.00506.x. PMid:15347395.

Medeiros MA, Barros ATM, Medeiros RMT, Vieira VD, Azevedo SS, Riet-Correa F. Sazonalidade da mosca-dos-chifres, *Haematobia irritans* no semiárido Paraibano. *Pesq Vet Bras* 2018; 38(7): 1307-1312. http://dx.doi.org/10.1590/1678-5150-pvb-5531.

Miraballes C, Marques AR, Medeiros MA, Barros ATM, Riet-Correa F, Gomes Lopes JR, et al. Data set for: identification of Sindhi cows that are susceptible or resistant to *Haematobia irritans. Mendeley Data* 2019; V2. http://dx.doi.org/10.17632/pwsgz5hp6p.2.

Miraballes C, Sanchez J, Barros ATM, Hitateguy S, Moreno P, Saporiti T, et al. Influence of selective treatment of bulls on the infestation of *Haematobia irritans* on untreated cows. *Vet Parasitol* 2018; 260: 58-62. http://dx.doi.org/10.1016/j.vetpar.2018.08.012. PMid:30197016.

Morrison DG, Foil LD. Effect of horn fly (Diptera: Muscidae) control during the spring on calf production by fall-calving beef cows. *J Econ Entomol* 1995; 88(1): 81-84. http://dx.doi.org/10.1093/jee/88.1.81. PMid:7884078.

R Core Team. *R: a language and environment for statistical computing* [online]. Vienna: R Foundation for Statistical Computing; 2013 [cited 2019 Apr 05]. Available from: http://www.R-project.org/

Sparks TC, Quisenberry SS, Lockwood JA, Byford RL, Roush RT. Insecticide resistance in the horn fly, *Haematobia irritans. J Agric Entomol* 1985; 2(3): 217-233.

Steelman CD, Brown CJ, McNew RW, Gbur EE, Brown MA, Tolley G. The effects of selection of size in cattle on horn fly population density. *Med Vet Entomol* 1996; 10(2): 129-136. http://dx.doi.org/10.1111/j.1365-2915.1996.tb00718.x. PMid:8744704.

Steelman CD, Gbur EE, Tolley G, Brown AH Jr. Individual variation within breeds of beef cattle in resistance to horn fly (Diptera: muscidae). *J Med Entomol* 1993; 30(2): 414-420. http://dx.doi.org/10.1093/jmedent/30.2.414. PMid:8459419.

Tugwell PE, Burns EC, Turner JW. Brahman breeding as a factor affecting the attractiveness or repellency of cattle to the horn fly. *J Econ Entomol* 1969; 62(1): 56-57. http://dx.doi.org/10.1093/jee/62.1.56.