International Journal of Agriculture Innovations and Research
Volume 3, Issue 2, ISSN (Online) 2319-1473
Manuscript Processing Details (dd/mm/yyyy) :
Received : 09/09/2014 | Accepted on : 21/09/2014 | Published : 26/09/2014

Building Quantitative Indicators for the Evaluating the Sanitary Condition of Cattle Herds

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***Abstract* – Aiming at developing a tool for assessing the magnitude of farm animal health, producing a quantitative indicator for evaluating the sanitary condition of cattle herds. To illustrate the construction of the indicator, serum samples were taken from 160 cattle from 16 properties in the island of São Luiz, state of Maranhão, Brazil, where four diseases were evaluated along with their impact percentages: infectious bovine rhinotracheitis, bovine viral diarrhea, bovine brucellosis and enzootic bovine leukosis – all of an infectious and transmissible nature and the cause of considerable economic losses. Disease frequencies were estimated and respective percentage impacts per herd were established The mathematical rationale underlying the building of the indicator ranks properties on a 0 - 100% scale in terms of disease positivity in the herd, with the lowest indicator value indicating the highest cumulative disease frequency (0% = all animals tested positive for the four diseases, 100% = all animals tested negative for all diseases). The "sanitary condition" of the herds ranged from 35% to 77.50%. Determination of the "sanitary condition" will directly impact the production chain and consumers, who will be able to ascertain animal health at the beginning of the production chain.**

***Keywords* – Cattle, Indicador, Infectious Diseases, Sanitary Status.**

I. INTRODUCTION

Health is a prerequisite for animal productivity in a production system. Absence of disease among populations, as well as being ideal, is a fundamental condition for avoiding interruption or breakage of productivity and the consequent cost increase. Occurrences of diseases contribute towards loss of value added to farms, with measurable losses to producers. Today, an attractive and safe environment that allows agribusiness to meet consumers' changing habits and increasing sanitary requirements is sought.

Among the main causes of losses in dairy cattle productivity are reproductive infections caused by bovine viral diarrhea (BVD), bovine herpesvirus type 1 (BoHV-1) and *Brucella abortus.* Infections caused by the enzootic bovine leukosis (EBL) virus can also reduce cattle productivity [1]. Studies confirm the high prevalence of these four diseases in the State of Maranhão [2, 3, 4, 5].

BoHV-1 is responsible for significant losses in cattle farming. Infection by this virus can result in a respiratory disease known as infectious bovine rhinotracheitis (IBR). As well as the respiratory symptoms, this virus induces conjunctivitis, infectious pustular vulvovaginitis (IPV), infectious pustular balanoposthitis (IPB), embryo reabsorption, abortion, temporary infertility, birth of weak animals and fatal multisystemic infection of new borns [6]. Infection by BVD can cause respiratory, digestive and reproductive clinical conditions, mucous disease (MD), hemorrhagic syndrome (HS) and immunosuppression. However, the greatest losses result from the infection of pregnant females, due to occurrences of stillbirth, fetal malformation and birth of weak calves that are persistently infected (PI) and immunotolerant to the virus [7, 8].

Brucellosis is one of the most important and widespread zoonoses in the world, according to the Food and Agriculture Organization (FAO), World Health Organization (WHO) and the World Organization for Animal Health (OIE) [9, 10, 11]. *Brucella abortus* is highly pathogenic and causes severe disease, especially among cattle [12]. This species also presents clinical and epidemiological importance and is considered pathogenic for humans [13].

Many kinds of losses are caused by bovine brucellosis. Herd present decreased milk and beef productivity; the market price of the animals and products of animal origin from endemic regions is diminished; the interval between births is increased, abortions occur among diseased females; sterility is observed; and it is recommended that positive animals should be culled, with the consequent expense of buying other animals to replace in the herd [14].

EBL is a neoplastic infectious-contagious, multi-symptom disease with chronic evolution that particularly affects the lymphoid cellular lineage of cattle. It is caused by the virus of bovine leukosis (VBL), a deltaretrovirus that is related to the T-cell lymphotropic viruses of primates [15].

The Terrestrial Animal Health Code of the OIE recommends that potential dangers that can be introduced to farms should be identified and that diseases that have already become established should be brought under control [16].

Efficient production of animals of zootechnical interest, with the capability to supply beef and milk, requires professional animal health planning at the origin of the production chain, with an integrated approach towards factors that interfere with production. This new vision makes it possible to integrate the production chain, thus doing away with the segmented vision of production. In this manner, products with greater market acceptance are

obtained, herd value is increased, trade barriers are overcome and industrial processes with standards allowing new consumer habits to be met become viable.

Disease control programs that indicate sanitary conditions and raise awareness among producers may be able to transform the animal product into a positive factor for animal and public health. In this context, the present study was conducted with the objective of producing a quantitative indicator for evaluating the sanitary condition of cattle herds.

II. Material and Methods

A. Study Area

This study was carried out on the island of São Luiz, state of Maranhão, comprising land in this state lying to the west of the 44° meridian. The study area consisted of the municipalities of Paço do Lumiar (area= 124.753 km^2; location: 44.1°S and 2.53°W), Raposa (area= 64.353 km^2; location: 44.1°S and 2.42°W), São José de Ribamar (area= 388.369 km^2; location: 44.05°S and 2.56°W) and São Luiz (area= 834.780 km^2; location: 44.3°S and 2.52°W) [17].

B. Sample Delineation

Sampling was conducted in two stages. First, the random selection of a predetermined number of properties, which represent the primary sampling units. Within the primary unit, were sampled at random a predetermined number of animals (slave units), in order to determine the health status of livestock. The calculation of the number of flocks was determined by the confidence level of the result, the level of accuracy desired and the amount of the expected prevalence [3], using the formula for a simple random samples to Thrusfield [18] and Noordhuizen et al. [19].

The random selection of herds for each municipality, was held from the register of existing properties at the base of the corresponding Local Veterinary Unit. For each municipality, the existing properties were numbered and the data stored in spreadsheets Microsoft Excel 2000 ® program.

The sample design for the secondary units aimed to estimate the minimum number of animals to be examined, within each property in order to enable its classification as a focus or not focus. The number of selected animals from each herd was determined using the Herdacc® program, *version 3* (University of Guelph), considering the aggregate values of sensitivity and specificity of diagnostic procedures, intra-herd prevalence and standard error.

The number of selected animals from each herd was similar, sampled 10 animals per property because the flocks were composed of up to 99 females [3, 20]. For the selection of animals within the property we used two methods, simple random sampling or systematic random.

A total of 160 serum samples from females reared in semi-intensive system of creating aged> 24 months from 16 herds not vaccinated against bovine viral diarrhea, infectious bovine rhinotracheitis, bovine enzootic bovine leukosis and brucellosis was analyzed.

C. Blood Sample Collection and Epidemiological Data

Blood was collected by means of jugular venipuncture after antisepsis, with the aid of a disposable 40 x 12 mm needle. The samples were kept in sterile test tubes, at rest and tilted to facilitate clot retraction, until laboratory processing. The tubes were then centrifuged for 15 minutes with a real centrifugation force of 2000G and the serum obtained was transferred to plastic tubes stored at -20° C.

At the time of sample collection, an epidemiological questionnaire was applied to obtain information about the farms, animals studied and sanitary management practiced, such as: herd size, veterinary care, vaccination and occurrences of reproductive, respiratory and digestive signs.

This work was approved by the Ethics Committee on Experimentation Animal in the Veterinary Medicine State University of Maranhão, protocol n. 037/2011.

D. Diagnostic Tests

The serological analyses were carried out in the Diagnostic Laboratory for Infectious Diseases of the Veterinary Course of the State University of Maranhão (UEMA).

D.1 BVD and BoHV-1

Qualitative detection of anti-BVD and anti-BoHV-1 antibodies was carried out using the ELISA technique, using a commercial indirect ELISA kit (CHEKITIBR – SERO, Dr. Bommeli AG/Liebefeld, Bern, Switzerland).

D.2 Enzootic bovine leukosis (EBL)

The serum samples were examined by means of Ouchterlony radial double immunodiffusion, which is recognized worldwide for detection of specific serum anti-VEBL antibodies. A gelatinous diffusion substrate was used, with a glycoprotein antigen (gp 51) extracted from the EBL virus envelope, which was produced by the Technological Institute of Paraná (TECPAR). The readings were made 72 hours after assembling the system.

D.3 Brucellosis

The buffered acidified antigen (BAA) test was used as a screening method for detecting anti-*Brucella abortus* antibodies, using an antigen produced by TECPAR.

The samples that were reactive to the BAA were simultaneously subjected to 2-mercaptoethanol (2-ME) and slow tube agglutination (STA), using an antigen produced by TECPAR, at the titrations of 1:25, 1:50, 1:100 and 1:200. The results were interpreted in accordance with the current legislation [21].

E. Metric of the Sanitary Condition Indicator

Considering, as a generalization, that N diseases were selected for studying the sanitary condition, each animal in the herd was evaluated regarding the frequency (positive findings) of the N diseases, thus resulting in several possibilities for occurrence combinations in each herd, with different percentages of these possibilities. The total number of possibilities consisted of several combinations of many answers, i.e. 2^N groupings, for which the results could range from negative findings for all animals to full presence of all the diseases among all animals.

In this study, N was four diseases (N = 4), so there were 16 possibilities in the herd: i) animals that were negative for all four diseases; ii) positive for BoHV-1; iii) positive for BVD; iv) positive for VEBL; v) positive for *Brucella abortus*; vi) positive for BoHV-1 and BVD; vii) positive for BoHV-1 and *B. abortus*; viii) positive for BoHV-1 and VEBL; ix) positive for BVD and *B. abortus*; x) positive for BVD and VEBL; xi) positive for *B. abortus* and VEBL; xii) positive for BoHV-1, BVD and *B. abortus*; xiii) positive forBoHV-1, BVD and VEBL; xiv) positive for BoHV-1, *B. abortus* and VEBL; xv) positive for BVD, *B. abortus* and VEBL; and xvi) positive for all four diseases.

To correlate the growing order of magnitude of the indicator with better sanitary condition among the herds, weights from 1 to N + 1 were attributed, according to the groupings of disease occurrences in the herd and according to the study of Padovani et al. [22]. The greater the weight received by the grouping was, the better the sanitary condition was, regarding the disease occurrence and, on the other hand, the smaller the weight was, the worse the sanitary condition was.

The groupings were made taking into consideration the number of disease occurrences among the animals, which determined N+1 distinct groups. Thus, a score (weight) of 1 was attributed to the group of animals that presented all the diseases, i.e. with N diseases (the most unfavorable situation for the indicator, and therefore the smallest weight). A score of 2 was attributed to the group of animals with (N – 1) diseases, and so on, until reaching the score of (N + 1), which was attributed to the animals that were disease-free (the most favorable situation for the indicator, and therefore the highest weight).

For the numerical calculation of the indicator of sanitary condition in each herd, the weighted total of the points obtained (WT) was initially considered, which was expressed by:

WT = (N + 1) * % disease-free animals (F_0) + N * % animals with one disease (F_1) + (N – 1)% animals with two or more diseases (F_2) +... + 2 * % animals with (N – 1) diseases (F_{N-1}) + 1 * % animals with the N diseases (F_N), thus:

$$WT = (N + 1) * F_0 + N * F_1 + (N - 1) * F_2 + ... + 2 * F_{N-1} + 1 * F_N$$

Where: WT = weighted total

F_0, F_1, ... F_N are the frequencies of positive animals for each herd group, specified as follows:

F_0 = frequency of negative animals (negative group);

F_1= frequency of animals with one of the diseases (positive for one disease group);

F_2 = frequency of animals with any two diseases (positive for two diseases group);

F_N = frequency of animals with all diseases (positive for R diseases group).

From the WT, the sanitary condition (SC) of each animal was established, such that the SC% value consisted of standardization of the WT on a percentage scale from 0 to 100%. On this scale, 0% was the most unfavorable situation for herd sanitary condition, in which N diseases were found simultaneously among all animals, and 100% was the most favorable situation, in which there was a herd with total absence of diseases among all animals. The intermediate values were obtained following the expression:

SC (%) = 100 * (WT – minimum score) / (maximum score – minimum score).

Therefore:

SC (%) = 100 * (WT – 100) / (100N) = (WT – 100)/N

To calculate sanitary condition, the following items were taken into consideration:

1) For each herd, the frequency of animals was determined as none, one, two, three or four occurrences of positive findings (F_0, F_1, F_2, F_3, F_4);

$$F\ (\%) = \frac{\text{Number of positive animals}}{\text{Total number of animals per herd}} \times 100$$

Therefore:

$$F\ (\%) = \frac{\text{Number of positive animals}}{10} \times 100$$

2) For each group of positive occurrences, weights ranging from 1 to 5 (N + 1 = 5) were established. The value of 5 indicated no occurrences of diseases, 4 indicated one occurrence, 3 indicated two occurrences, 2 indicated three occurrences and 1 indicated all four occurrences;

3) Based on the weights and the respective frequencies of disease combinations in the herd, the total number of points was determined by the expression:

$WT = 5 * F_0 + 4 * F_1 + 3 * F_2 + 2 * F_3 + 1 * F_4$

4) The value corresponding to the "sanitary condition" was obtained from the score through the following expression:

SC (%) = (WT – 100)/4

5) The resultant value between 0 and 100% indicated the "sanitary condition" of the herd: the greater the value was, the better the animal health quality was.

III. Results

Frequencies of 67.50%, 68.12%, 62.50% and 3.12% were found for infections by BVD, BoHV-1, EBL and *Brucella abortus*, respectively. Furthermore, combination percentages of 8.12% for no disease, 20% for one, 36.36% for two, 35 for three and 0.62% for all the diseases evaluated in the study were found.

Table I illustrates the "sanitary condition" attributed to each herd, according to the frequency of positive responses to BoHV-1, BVD, *Brucella abortus* and EBL.

Table I: Sanitary condition of 16 herds on the island of São Luís, Maranhão, according to the frequency of positive responses to BoHV-1, BVD, *Brucella abortus* and EBL diseases

Municipality	Herd	Frequency of disease combinations (%)					WT (%)	SC (%)
		F_0	F_1	F_2	F_3	F_4		
Paço do Lumiar	1	10	20	40	20	10	300	50
	2	10	20	30	40	0	300	50
	3	40	40	10	10	0	410	77.5
	4	10	20	50	20	0	320	55

São Luís	5	20	20	40	20	0	340	60
	6	0	10	60	30	0	280	45
	7	10	10	40	40	0	290	47.5
	8	20	20	30	30	0	330	57.5
São José de Ribamar	9	0	10	50	40	0	270	42.5
	10	0	20	50	30	0	290	47.5
	11	0	50	20	30	0	320	55
	12	0	50	40	10	0	340	60
Raposa	13	0	0	40	60	0	240	35
	14	0	20	10	70	0	250	37.5
	15	0	10	40	50	0	260	40
	16	10	0	30	60	0	260	40
Weight of the occurrences		**5**	**4**	**3**	**2**	**1**		

On the scale from 0 to 100%, there was a range of sanitary conditions among the herds. The smallest attributed value was 35% and the highest was 77.50% among the 16 farms evaluated.

IV. Discussion

The results found demonstrated that BoHV-1, BVD and VEBL were present at high percentages among the cattle herds evaluated, which characterizes a very important sanitary problem. Despite the low frequency of anti-*Brucella abortus* antibodies found, this value was equivalent to official data that indicate that the prevalence of animals that are seropositive for brucellosis is between 4 and 5% [23]. The frequency values found in the present study and other [2, 3, 4, 5] justify the choice of these diseases to illustrate the compilation of the indicator of sanitary condition for cattle by means of mathematical modeling.

The diseases among the population that were identified in this study may influence productivity among the herds through reducing it and not allowing the animals to reach their maximum performance and economic profitability. This obstacle can be aggravated by associations between the diseases studied. Additionally, host stress due to productive life (lactation and maximization of weight gain) has an important role in triggering infectious diseases, with a clinical-immune condition that includes BVD and EBL and their wide dissemination among cattle herds [24].

Studies have indicated that BVD causes immunosuppression among infected animals and increases their susceptibility to other opportunistic viruses, such as BoHV-1. The immunosuppression caused by BVD can be correlated with the tropism of this virus for cells of the immune system, such as CD4+ T cells and CD8+ T cells, B cells, monocytes, macrophages and dendritic cells [25]. In addition, BVD has been reported to modulate the functions of immunological cells after *in vitro* infection, with increased production of nitric oxide in infected macrophages, decreased production of tumor necrosis factor α (TNFα), reduced expression of Fc receptors and complement proteins (C3) and reduced phagocytic activity of alveolar macrophages [26].

The role of EBL in triggering of opportunistic bacterial diseases of clinical-epidemiological and public health importance is unknown. However, it is known that the compromising of the integrity of the immunological system through the immunosuppressive action of EBL, which penetrates and is incorporated in the lymphocyte genome for an indeterminate length of time, together with immunocytological evidence that it infects circulating monocytes, increases host susceptibility to other infections [27].

With the sanitary condition indicator, it was possible to identify the six herds (3, 4, 5, 8, 11 and 12) that presented the best sanitary condition among the 16 herds evaluated, with values that ranged from 55 to 77.5%. Herd 3 (SC = 77.50%) and herd 4 (SC = 77.50%) were found to be receiving regular veterinary care. For the other herds, the absence of veterinary medical, joint creation of goats, pigs and sheep and frequent purchase of cattle from non reputable properties care may have been reflected especially in the diagnosis and in the absence of control programs for the diseases studied, as well as contributing towards worse sanitary conditions on these farms.

The sanitary condition indicator obtained through mathematical modeling that is proposed in this study can be applied to different epidemiological situations (countries) that involve a higher or lower number of diseases, co-diseases and correlations between diseases, among other situations. It can also be applied to different types of animal breeding (sheep, goats, buffaloes, horses, pigs, poultry, etc.), with higher or lower numbers of farms, as well as to serum samples.

With the advance of agribusiness and globalization, changes in sanitary requirements and consumer habits have occurred. These factors show that there is a need for better integration of the production chain, which can express the sanitary quality of farms and consequently of their animal herds.

Mathematical modeling has been shown to be an important tool for decision-making on issues that involve breeding, and has produced efficient results over a short time scale. Modeling allows integration of knowledge from wide variety of sources relating to the issue at hand and, through this, studies can have broad reach. However, for the results produced by mathematical simulation to be reliable, it is necessary to supply trustworthy information. Thus, the success of mathematical modeling depends on the results from traditional experimentation.

V. Conclusion

Based on the results obtained, it can be concluded that this modeling of sanitary condition may constitute an alternative that can help in decision-making and in defining better and more economical products, both within industrial production and for research. This methodology seeks to transform pertinent data, concepts and knowledge into mathematical equations and implement them by means of logical processes. Thus, the sanitary information from farms and research institutions can be applied to evaluate animal herds and define animal health priorities.

Therefore, construction of a quantitative indicator to express sanitary conditions can contribute towards evaluate animal health at the beginning of the production chain, on farms, thereby directly reflecting the quality of the commercialized product with a positive market impact, and at the end of the chain, with the final consumers as the target, allowing them to have the possibility of identifying the sanitary condition of the herds.

ACKNOWLEDGMENT

The Foundation for Scientific Research and Development of Maranhão (FAPEMA) and State University of Maranhão (UEMA) for making this study possible.

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