Journal of Animal and Veterinary Advances 16 (3-7): 69-74, 2017
ISSN: 1680-5593

An Assessment of Discriminatory Power of Postmortem Lesions for Diagnosis of Salmonellosis in Field Condition in Laying Flocks

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Abstract: Avian Salmonellosis is considered as one of the major diseases in layer industry. In resource-limited condition diagnosis of Salmonellosis is based on subjective postmortem examination. Discriminatory power of this subjective diagnosis has not been systematically examined before. Thus, the present study examined the discriminatory power of the postmortem lesions for diagnosing Salmonellosis in the layer flocks. Multivariable diagnostic model based on postmortem lesions was developed to diagnose Salmonellosis in layer chicken. Post-mortem examinations of 430 dead chickens have been performed of which 138 (32.09%) were found Salmonellosis positive based on the postmortem lesions. The 17.4% (24 out of 138) of postmortem suspects were confirmed Salmonella positive by laboratory tests. In univariable analysis enlarged liver, bronze liver, enlarged spleen, oophoritis, haemorrhage in the intestine and necrotic foci in the liver all produced a $p = 0.1$ and were included in the multivariable model. The multivariable diagnostic model was developed using logistic regression and backward elimination procedure. Enlarged liver, bronze liver, enlarged spleen, oophoritis, the haemorrhage in the intestine were found significant ($p = 0.05$) in the final model. Hosmer and Lemeshow goodness of fit statistics for the final diagnostic model had a p-value estimate of 0.544 which indicates a good model fit. The area under the ROC curve was 0.826 which shows moderately high diagnostic discriminatory power. As the postmortem lesions demonstrated a moderately high discriminatory power to differentiate Salmonella positive birds from negative birds, postmortem lesions based diagnosis of Salmonellosis could be considered as a useful approach in resource-limited countries where access to the diagnostic laboratory is limited.

Key words: Salmonellosis, layer chicken, post-mortem lesions, objective diagnosis, discriminatory power, approach

INTRODUCTION

Due to the consumer demand, a tremendous growth has been observed in the poultry industry throughout the world (Yegani *et al*., 2005a, b).This growth has come with several challenges-namely, increased density of poultry population, rearing them in proximity as well as a dramatic change in genetic potential at the cost of the fragility of the poultry against diseases that requires improved management practice (Yegani *et al*., 2005a, b). Thus, disease problem popped up as a major issue in the modern poultry industries (Yegani *et al*., 2005a, b). Avian Salmonellosis considered as one of the major economic diseases for poultry industry globally (Bouzidi *et al*., 2012). The magnitude of Salmonellosis in the layer farms in Bangladesh is also high (Islam *et al*., 2006; Hasan *et al*., 2012).Central to any discussion of the diseases like Salmonellosis in connection with the magnitude of a burden as well as prevention and control is the diagnosis. Diagnostic information plays an integral role in making a diagnostic decision and disease management.

Field skills (clinical and postmortem examination) and laboratory procedures have been emphasized for making a diagnosis of poultry diseases (Bagust, 2013a, b;

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Yegani *et al*., 2005a, b). However, resource-poor situation limits the laboratory diagnosis of the infectious diseases. Thus, field skills are of particular importance for the diagnosis of the infectious diseases in resource-limited countries (Catley *et al*., 2012). Bangladesh is a resource-poor country and diagnosis of poultry diseases is based on post-mortem examinations (Hasan *et al*., 2012). Necropsy of poultry and systematic examination of postmortem lesions provides an immediate tentative diagnosis of poultry diseases (Bagust, 2013a, b; Yegani *et al*., 2005a, b). It has also been demonstrated earlier that postmortem examination is one of the best tool for detection of Salmonellosis in layer flocks (Hoorebeke *et al*., 2009).

Several researcher have been described individual postmortem lesions of Salmonellosis in chicken (Basnet *et al*., 2008; Chanie *et al*., 2009; Ezema *et al*., 2009; Khan *et al*., 2004; Jones *et al*., 2001; Kumari *et al*., 2013; Majid *et al*., 2000; Oh *et al*., 2010; Srinivasan *et al*., 2014). Postmortem lesions might be useful for making a valid diagnosis of Salmonellosis in layer chicken. However, the discriminatory power of diagnosing Salmonellosis in layer chicken based on postmortem lesions has not systematically been examined earlier. Moreover, researchers have been attempted to describe the postmortem lesions subjectively and seem to base on their clinical experience. Thus, the diagnostic merit of different postmortem lesions is not quantitatively known so far. Although, useful, the subjective analysis is often not sufficient for making a definitive diagnosis and need to be supplemented with objective methods (Bankier *et al*., 1999).

Objective analysis approaches like multivariable logistic regression model can quantify the discriminatory power of the postmortem lesions. For binary outcome like disease present or absent, logistic regression has become themethod of choice (Hosmer *et al*., 2013). Application of multivariable diagnostic model is common in human clinical setup (Cook, 2008). Although, less frequently used in veterinary medicine but not merely absent in veterinary medicine, for example, a model for discriminating septicemia in calves (Lofstedt *et al*., 1999). Objective approaches like logistic regression model control the inherent problem of arbitrary discrimination of subjective analysis in assessing diagnostic merit. The logistic regression model can take account of the effect of each predictor and can adjust for the presence of researcher predictor in the model and any interaction between the independent predictor can also be included. A ranking order of the predictors based on their ability to discriminate positive and negative cases can also be obtained (Hosmer *et al*., 2013).

The purpose of the present research was to provide field veterinarians with a set of postmortem lesions with known discriminatory power for the diagnosis of Salmonellosis in layer chicken in field condition of resource-limited countries with a greater accuracy. The objective of the study was to develop a multivariable diagnostic model using logistic regression based on individual postmortem lesions of Salmonellosis in layer chicken.

MATERIALS AND METHODS

Literature study and sample collection: The 430 dead chickens from the laying flocks of Pahartoli Government Zonal Poultry Farm, Chittagong and Bangladesh were subjected to postmortem examination. Postmortem examination has been performed by a panel of three veterinarians from Teaching Veterinary Hospital of Chittagong Veterinary and Animal Sciences University (CVASU). The panel looked for the postmortem lesions have been found useful for the diagnosis of Avian Salmonellosis from the literature review (Table 1). Liver, heart, lung, spleen, proventriculus, intestine and caeca were examined carefully and lesions found were recorded. The diagnosis was made by the panel while they reached the consensus by the performed postmortem examination. A liver sample from the suspected positive case was then sent to the Microbiology Laboratory of CVASU for bacteriological examinations.

Sample isolation: The liver samples from suspected cases of Salmonellosis were used to make swab for inoculation. The surface of the liver sample was seared with a hot scalpel and subsequently, anincision was made over the seared area to collect inoculum with inoculating loop. The sample obtained by inoculating loop was streaked on blood agar base enriched with sheep blood and incubated up to 48 h bacteria on culture positive plates were subjected to gram staining. Gram-negative bacteria from positive culture plates were taken on McConkey agar plate and streaking was made properly by using an inoculating loop. All inoculation work was done in a laminar flow to avoid the chance of contamination. Then the Petri dishes were incubated at the inverted position for 24 h at 37°C temperature. Clear and transparent colonies were considered as Salmonella colonies. The positive colonies were transferred into the nutrient broth and incubated for 24 h at 37°C temperature for the growth of pure culture and better multiplication of the organisms. For more definitive diagnosis by biochemical test, Salmonella suspects were inoculated to the slant of Triple Sugar Iron (TSI). TSI slants were

Table 1: Previously reported individual postmortem findings determined to be diagnostically useful for avian Salmonellosis in chicken

Postmortem findings	Researchers
Enlarged liver	Majid *et al.* (2000), Jones *et al.* (2001), Khan *et al.* (2004), Basnet *et al.* (2008), Chanie *et al.* (2009), Ezema *et al.* (2009), Kumari (*et al.* (2013) and Srinivasan *et al.* (2014)
Metallic sheen of liver (bronze liver)	Majid *et al.* (2000), Jones *et al.* (2001), Khan *et al.* (2004), Basnet *et al.* (2008) and Kumari *et al.* (2013)
Necrotic foci in liver	Majid *et al.* (2000), Jones *et al.* (2001), Khan *et al.* (2004), Basnet *et al.* (2008), Chanie *et al.* (2009), Ezema *et al.* (2009) and Oh *et al.* (2010)
Enlarged spleen (marble shape spleen)	Majid *et al.* (2000), Jones *et al.* (2001), Khan *et al.* (2004), Basnet *et al.* (2008), Chanie *et al.* (2009) Ezema *et al.* (2009), Kumari *et al.* (2013) and Srinivasan *et al.* (2014)
Haemorrhage in intestine	Majid *et al.* (2000), Jones *et al.* (2001) and Kumari *et al.* (2013)
Syanovitis	Oh *et al.* (2010)
Oophoritis (inflamed, discoloured and misshapen ovary)	Khan *et al.* (2004), Chanie *et al.* (2009), Ezema *et al.* (2009) and Srinivasan *et al.* (2014)
Enlarged kidney (swollen kindney)	Majid *et al.* (2000), Jones *et al.* (2001) and Kumari *et al.* (2013)
Pericarditis	Majid *et al.* (2000), Ezema *et al.* (2009), Oh *et al.* (2010), Kumari *et al.* (2013) and Srinivasan *et al.* (2014)

incubated for 24 h at 37°C temperature. Alkaline (red) slant and acidic (yellow) but with blackening of media due to the production of H_2S were considered for the Salmonella positive samples. Data on a positive culture and biochemical test were recorded for corresponding positive samples from postmortem suspects.

Sample analysis: The dependent variable of the diagnostic model was defined based on the bacteriological examination. Culture and TSI positive samples from the postmortem examination based suspected cases were regarded as Salmonella positive. All other suspected samples were considered as Salmonella negative. Individual postmortem lesions recorded during postmortem examination by the panel of a veterinarian for suspected cases were used as the independent variable of the diagnostic modelling. The model-building procedure was initiated by screening postmortem lesions by univariable analysis. Chi-square statistic was applied for this purpose. Independent postmortem lesions with $p \leq 0.1$ were considered further and were incorporated into a stepwise backward logistic regression procedure to develop a multivariable diagnostic model for Salmonellosis in layer chicken. Interaction and confounding were checked during the model building process. The goodness of the fit model was evaluated based on Hosmer and Lemeshow (1980) goodness of fit chi-square statistics. All the data analysis procedure was conducted in STATA 12. The accuracy of the logistic model in discriminating Salmonella positive and negative cases was evaluated using the area under a receiver operating characteristic curve.

RESULTS AND DISCUSSION

Confirmed Salmonella samples: Of 430 dead birds subjected to postmortem examination a total of 138 (32.09%) birds were diagnosed as suspected positive cases by the panel of the veterinarians. Culture and TSI results were available for all suspected cases. Based on the culture and biochemical tests 24 (17.4%) samples were confirmed as Salmonella positive.

Multivariable diagnostic model for Salmonella positive samples: The univariable analysis examined postmortem lesions that are potentially useful for diagnosis of Salmonellosis in layer chicken and results were presented in Table 2. Six of the ten postmortem lesions yielded a $p<0.10$ and were subsequently included in the multivariable analysis. Univariable analysis revealed that bronze liver ($p = 0.039$), enlarged liver ($p = 0.002$), enlarged spleen ($p = 0.004$), hemorrhage in the intestine ($p = 0.051$), oophoritis ($p = 0.056$) and necrotic foci in liver ($p = 0.027$) were more likely to present with laboratory-confirmed Salmonella positive group than the negative group.

The results of the multivariable diagnostic model are shown in Table 3. Five postmortem lesions remained significant in the final diagnostic model. Enlarged liver, bronze liver, enlarged spleen, haemorrhage in intestine andoophoritis were identified as significant postmortem lesions by multivariable analysis for in diagnosing Salmonellosis in layer chicken. There was no confounder and no interaction term was found significant during the model building process. Hosmer and Lemeshow (1980) goodness of fit Chi-square statistics for the final diagnostic model had a p-value estimate of 0.544. ROC curve assessed the discriminatory power of the model (Fig. 1) and the area under the ROC curve was 0.826.

In this study, we developed a multivariable diagnostic model, based on postmortem lesion for the diagnosis of Salmonellosis in laying flocks. A multivariable diagnostic model is an objective approach that can provide information on the discriminatory power of the diagnosis and relative importance of the individual postmortem lesions. The model identified enlarged liver, bronze liver, enlarged spleen, intestinal hemorrhage and oophoritis are significant postmortem lesions for diagnosing Salmonellosis and are able discriminate

Table 2: Results of univariable analysis of postmortem findings

Postmortem findings	Salmonella positive N = 24 (%)	*Salmonella* negative N = 114 (%)	χ^2 (p)
Enlarged liver	16 (66.7)	38 (33.30)	9.249 (0.002)
Metallic sheen of liver (bronze liver)	15 (62.5)	45 (39.50)	4.278 (0.039)
Necrotic foci in liver	4 (16.7)	5 (4.38)	4.095 (0.027)
Enlarged spleen (marble shape spleen)	15 (62.5)	36 (31.60)	8.136 (0.004)
Haemorrhage in intestine	14 (58.3)	42 (36.80)	3.798 (0.051)
Syanovitis	5 (20.8)	15 (13.20)	0.943 (0.332)
Oophoritis (inflamed, discoloured and misshapen ovary)	6 (25.0)	12 (10.50)	3.662 (0.056)
Enlarged kidney (swollen kidney)	18 (75.0)	73 (64.00)	1.061 (0.303)
Pericarditis	3 (12.5)	12 (10.50)	0.080 (0.778)

N, number; p, p<0.1 indicate significance

Table 3: Multivariate predictors of *Salmonellosis* with odds ratios and their statistical significance

		CI 95%		
Postmortem findings	OR	Lower	Upper	p-values
Oophoritis	6.203	1.607	23.9370	0.008
Enlarged spleen	5.181	1.743	15.3960	0.003
Enlarged liver	3.499	1.199	10.2040	0.022
Bronze liver	3.337	1.084	10.2670	0.036
Hemorrhage in intestine	3.000	1.057	8.5110	0.039

OR: Odds Ratios: CI, Confidence Interval

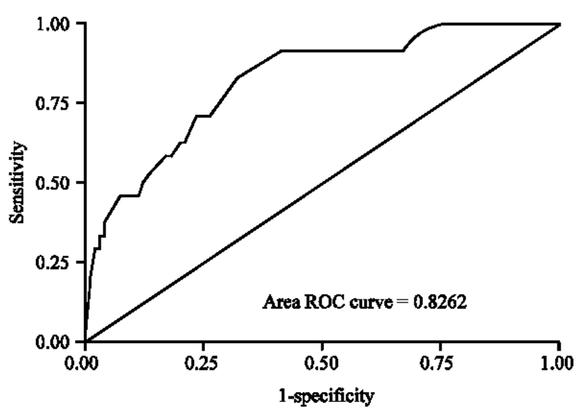

Fig. 1: Receiver operating characteristic curve for the diagnostic model developed by logistic regression model

laboratory-confirmed Salmonella positive birds from Salmonella negative birds. In other word, birds with postmortem lesions of enlarged liver, bronze liver, enlarged spleen, intestinal hemorrhageand oophoritis have a higher chance of being diagnosed as Salmonella positive while subjected to bacteriological examinations. The postmortem lesions found significant in the final diagnostic model are biologically plausible because they are in line with the typical and classical gross pathological lesion described earlier for Salmonellosis in layer chicken (Jones *et al*., 2001). The relative importance of the individual postmortem lesions found significant in the model can be assessed by their respective Odds Ratios (OR). The ranking order of relative importance of postmortem lesions based on OR of the (Table 2) for discriminating Salmonella cases are oophoritis, enlarged spleen, enlarged liver, bronze liver and hemorrhage in the intestine.

It is always important to assess how well the logistic regression model fits the observation during the model building process (Hosmer *et al*., 2013). Hosmer and Lemeshow (1980) described a goodness of fit based on Chi-square statistics. A lower p-value (p<0.05) of H-L Chi-square statistics indicates poor fit of the model; conversely higher p-value shown the better fit of the model (Hosmer and Lemeshow, 1980; Hosmer *et al*., 2013). The H-L goodness of fit Chi-square statistics for the developed diagnostic model had a p-0.544, indicates that the model developed in this study fits the postmortem lesions reasonably well for diagnosing Salmonellosis. Besides, the diagnostic model developed in this study has an AUC of 0.826 indicates that the discriminatory power of the model is moderately high. ROC plot is a statistical way of measuring the diagnostic accuracy (Cook, 2008; Soreide, 2009). The closer the curve to the diagonal, the diagnostic accuracy of the model is reduced. Further, the curve moves to the left or right of the diagonal the accuracy of the diagnosis increases. A value over the distance is measured by area under the ROC curve (AUC) (Soreide, 2009). More clearly, an AUC 0.5 indicates an inability of the model to differentiate diseased and from the no diseased. An AUC value greater or <0.5 indicates that the model can discriminate the outcomes.

Duration of the disease episode and previous treatments could have produced a difference in the postmortem findings and laboratory data between Salmonella positives and Salmonella negatives. Further, the present study did not examine the difference of pathological findings produced by other systematic bacterial infections like Staphylococcus, *E. coli*, Pasteurella infections and so on. These pathogens could produce similar postmortem lesions while causing systemic diseases in the layer flocks. Thus, we have used selective media and biochemical tests suggested by OIE (World Animal Health Organization) for laboratory confirmation of Salmonellosis in poultry. Besides, there is a chance of observation bias that generated by the veterinarians performed diagnosis based on postmortem findings. We believe that these influences will continue to be same in a future population where this model could be used. Thus, the model will remain equally valid for the diagnosis of Salmonellosis in laying flock in field condition of resource-limited countries. However, we suggest a validation of the model before it could be used on a new population of interest keeping earlier describe limitations in mind.

The confirmatory diagnosis of any disease by the laboratory is not always possible and feasible for the field veterinarians due to resources and time constraint (Catley *et al*., 2012). In resource-poor condition, poultry veterinarians have to rely on postmortem examination for diagnosing poultry diseases. Also, no laboratory methods ensure perfect test result every time for diagnosis of poultry diseases (Yegani *et al*., 2005a, b). Moreover, birds tentatively considered Salmonella infected, but not positive in confirmatory diagnosis resulting in excess cost and additional time. As the postmortem lesions demonstrated a moderately high discriminatory power to differentiate Salmonella positive birds from negative birds, postmortem lesions remain useful for the poultry veterinarians in field condition for diagnosing Salmonellosis in layer flocks.

CONCLUSION

The confirmatory diagnosis of poultry disease by the laboratory is not always possible and feasible for the field veterinarians due to resources and time constraint in resource-limited countries. Thus, veterinarians rely on clinical and postmortem examinations for diagnosing poultry diseases like Salmonellosis. However, this form of diagnosis is subjective and the discriminatory power is not known. In the absence of objective approaches for diagnosing Salmonellosis in field condition of resource-limited countries, we developed a multivariable diagnostic model based on postmortem lesions only. This model is based on simple logistic analysis and able to describe the discriminatory power of the diagnosis. Therefore, will useful information to the veterinarians in diagnosing Salmonellosis in layer chicken with a greater accuracyby using postmortem lesions only. The approach we used is generic and can be used for other diseases in a resource-limited situation.

ACKNOWLEDGEMENTS

The researchers thank panel of veterinarians from Teaching Veterinary Hospital of Chittagong Veterinary and Animal Sciences University (CVASU) to perform post-mortem examination and to the Microbiology Laboratory of CVASU for bacteriological examinations.

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