A Short Note: A study in determining the correlation between cortisol concentration in vaccinated and non-vaccinated clinically infected pneumonic goats

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Abstract
In Malaysia, pneumonic pasteurellosis is one of the major problems in the small ruminant industry. Small ruminants especially goats are more susceptible to the disease that usually occurs due to stressful conditions. Cortisol plays an important role in the disease development of pneumonic pasteurellosis. The main objective of this study was to determine the correlation between cortisol concentration in vaccinated and non-vaccinated pneumonic goats. A total of 15 normal goats and 9 clinically infected pneumonic goats were selected from 2 vaccinated farms, whereas 15 normal goats and 31 clinically infected pneumonic goats from 2 non-vaccinated farms were selected for this study. Goats with clinical signs of nasal discharges, coughing with crackle lung sound were diagnosed as pneumonic goats. Blood samples were collected from the selected goats for cortisol evaluation. The study revealed that non-vaccinated clinically infected pneumonic goats exhibited a slightly higher ($p>0.05$) cortisol value compared to the other experimental goats. Therefore, it can be concluded that the use of plasma cortisol concentration as a measure of stress contributing towards pneumonic pasteurellosis in goats might not be the best way to confirm the influences of the disease.

Keywords: Correlation, cortisol, vaccinated, non-vaccinated, clinical, pneumonic, goats.

Introduction

The small ruminant sub-sector in Malaysia is very small compared to the poultry and swine industry; and mainly operated by smallholder farmers (Loh, 2004). In 2016, the populations of sheep and goat consisted of only 145,999 and 431,651 heads, respectively (Department of Statistics Malaysia, 2017). The per capita consumption (PCC) of mutton was only at 1.2 kg/year for the year 2016 and the self-sufficiency level (SSL) of mutton in Malaysia was 11.8%. Therefore, the local mutton market is heavily dependent on the importation of mutton and chevon from other countries. The lag in the sheep and goat industry is normally associated with several factors such as the lack of land resources, high feed price, cheaper mutton and lamb import substitutes, poor private-sector involvement, inefficient disease prevention and control and lack of quality breeds, expertise, and workforce (Loh, 2004).

Pneumonic pasteurellosis caused by *Mannheimia haemolytica* is one of the major disease-related problems in Malaysia. The severity of the disease greatly led to the reduction in the number of small ruminants in Malaysia (Sabri *et al.,* 2013). The

incidence rate of pneumonia in ruminants ranged from 10 to 40% with mortality exceeding 20% where these percentages were higher in young ruminants (Rico *et al.*, 2017). The disease in its typical clinical form is highly infectious, acute febrile progression with severe fibrinopurulent bronchopneumonia and septicaemia. Disease ridden animals may die in a few days of clinical manifestations, but survivors from the bacterium may become persistently infected (Emikpe *et al.*, 2013). Although vaccination is the best preventive measure against pneumonic pasteurellosis, the disease is still very common in Malaysia. This is due to the unpopular usage of pasteurellosis vaccine among small ruminant farmers (Jesse *et al.*, 2018).

Cortisol plays an important role in the disease development for pneumonic pasteurellosis. Stressors such as climate, poor nutrition, poor husbandry and mainly transportation cause immunosuppression that contributes to the development of pneumonic pasteurellosis in sheep and goats leading to huge economic losses (Chung *et al.*, 2015). Cortisol is a corticosteroid, specifically within the glucocorticoid group, and is one of the stress hormones, other than catecholamines, produced by adrenal glands. Glucocorticoids serve many functions, including gluconeogenesis, lipolysis, block inflammatory response and inhibition of protein synthesis (Klein, 2013). The hormone is also associated with suppression of the immune system when there is an increase in the cortisol level (Tizard, 2013). There is a gap of knowledge related to the cortisol influence towards occurrence of this disease clinically for vaccinated and non-vaccinated goats. Thus, the aim of this study was to determine the correlation between cortisol concentration in vaccinated and non-vaccinated clinically infected pneumonic goats.

Materials and Methods

Experimental design

This study was conducted with the approval of the Institutional Animal Care and Use Committee UPM/IACUC/AUP-U011/2018. A total of 15 clinically normal healthy goats and 9 clinically infected pneumonic goats were selected from 2 vaccinated farms, while 15 clinically normal healthy goats and 31 clinically infected pneumonic goats from 2 non-vaccinated farms were selected for this study. The age of the goats included in this study were within one to three years of age. Goats with nasal discharges and signs of coughing were diagnosed as pneumonic goats. Lung auscultation was performed thoroughly on the thoracic region of each goat to confirm the crackle sound of pneumonia. Blood samples were collected from the selected goats via jugular venipuncture using 5 mL plain tubes for cortisol analysis.

ELISA analysis

The cortisol concentration (pg/ mL) was measured using enzyme-linked immunosorbent assay (ELISA) kits (Qayee-Bio, Shanghai). Firstly, the standard diluents were diluted accordingly before added to the standard wells. Then, 40 µL of special diluents were added into test sample wells followed by 10 µL of sample. Next, 50 µL of horseradish peroxidase (HRP) was then added to all wells except blank wells before sealing, gentle shaking and incubating for 60 min at 37°C. Excessive liquid was then discarded, dried, filled with washing liquid, mixed and shook for 30 min before discarding again and tapped into an absorbent paper to dry. This procedure was repeated for 5 times. After drying, 50 µL of chromagen solution A was added and followed with 50 µL of chromagen solution

B into all wells. The plate was gently shaken and incubated for 10 min at 37°C away from light. Finally, 50 µL of stop solution was added to each well to stop the reaction. The optical density (OD) values were measured at 450 nm wavelength using a BioRad ELISA reader. The result was determined by plotting the standard curve using the standard concentration and optical density values using the Expertcurve software.

Statistical analysis

JMP® Version 11. NC: SAS Institute Inc. software was used to analyse all data collected. ANOVA with control, Dunnett's test, were used to compare means between treatment groups. The data were considered significant at $p<0.05$.

Results and Discussion

Non-vaccinated clinically infected pneumonic goats exhibited a slightly higher cortisol concentration compared to the other experimental goats (Figure 1). However, the cortisol concentrations of the vaccinated normal goats, vaccinated clinically infected pneumonic goats, non-vaccinated normal goats and non-vaccinated clinically infected pneumonic goats were 17.74 ± 4.43 pg/mL, 19.69 ± 3.37 pg/mL, 17.79 ± 4.45 pg/mL and 22.98 ± 4.71 pg/mL, respectively, with no significant difference among experimental groups ($p>0.05$).

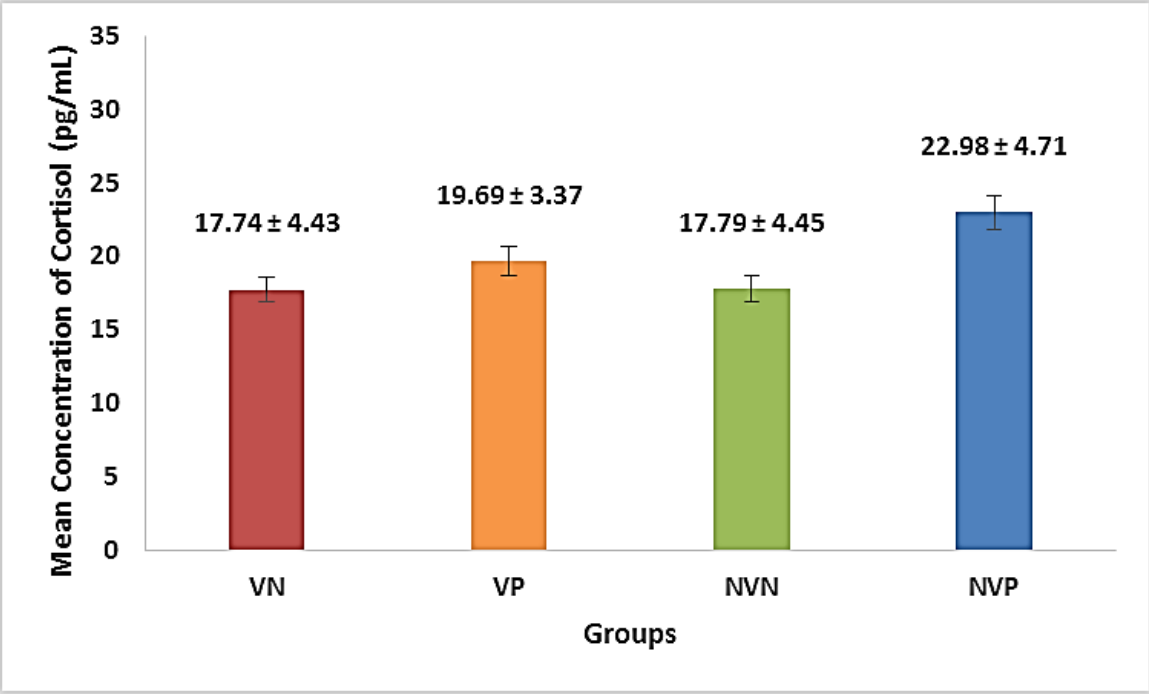

Figure 1: Mean concentration of cortisol in vaccinated and non-vaccinated goats
All values are expressed in mean ± SE, values with asterisk are significantly different at $p<0.05$
VN = Vaccinated Normal Goat, VP = Vaccinated Pneumonic Goat, NVN = Non-Vaccinated Normal Goat, NVP = Non-Vaccinated Pneumonic Goat.

Cortisol has been established as a good indicator of stress in farm animals (Olsson and Hybring-Sandberg, 2011). Among various stressors, bacterial infection is considered as a type of stressor which puts animals under stressful conditions (Abdelsalam, 2008). The present work was conducted to elucidate the correlation between cortisol concentration in normal and clinically infected pneumonic goats caused mainly by *M. haemolytica* in both vaccinated and non-vaccinated farms. Nonetheless, there was no significant difference in the cortisol concentrations among normal goats and pneumonic goats in both vaccinated and non-vaccinated farms. This finding was similar to Ganheim *et al.* (2003), where experimental calves infected with either bovine viral diarrhoea virus or *M. haemolytica.* exhibited no significant difference in the cortisol values. The evaluation of blood cortisol in chronically exposed stressors like pneumonic pasteurellosis may not be a reliable parameter to be studied in a naturally exposed herd probably due to the coping mechanism of the immune system towards the disease. However, immunosuppressed animals will have a higher risk of developing pathologies, reduced production, reproductive failure, reduced growth and even abnormal behaviour (Andersen *et al.*, 2008). Therefore, the cortisol level may be higher compared to healthy animals which were observed in this study.

In the current experiment, blood samples were only collected once where the pneumonic goats could be in different stages of the disease. Consequently, the cortisol levels obtained may not reflect the actual stress level of those experimental goats. Measurement of stress hormones such as cortisol is time-dependent where it takes approximately 15 to 20 min to reach its peak value after an animal is stressed and decreases over time (Grandin, 2000). This is supported by Miller *et al.* (1991), who found that cortisol concentration in experimentally induced chronic stress sheep rose temporarily before went back to a lower concentration. Consistent results were reported by Greenwood *et al.* (1992) and Ali *et al.* (2006), where cortisol concentration showed a significant increase after the exposure to acute stress of transportation. According to Gaughan *et al.* (2014), only initial stress challenge will significantly increase the expression of cortisol and no significant change seen in subsequent expression due to the acclimatization by the animals to the chronic exposure of stress.

The cortisol level in livestock falls into three basic categories: (1) resting baseline levels, (2) level provoked by being held or restrained and (3) excessive levels which are double or triple the restraint level (Grandin, 2000). In the present study, all experimental goats exhibited almost similar cortisol concentrations with no significant difference where the cortisol released may be provoked by restraining during blood sampling. It might have been even more reliable to use other measures of stress such as body fluids or excreta in contrast to blood. According to Ekiz *et al.* (2012), other non-invasive measures from the saliva, urine, faeces or hair are more reliable to measure the cortisol concentration in livestock. Plasma cortisol may only be a valuable indicator of stress in livestock under carefully controlled experimental conditions (Moberg and Mench, 2000). Besides, the evaluation of stress will be more accurate if behavioural reactions, heart rate, and other blood chemistries were also measured.

Conclusion

Therefore, the results from this study have concluded that there was no correlation between cortisol concentration in vaccinated and non-vaccinated clinically infected pneumonic goats. The use of plasma cortisol

as a measure of stress due to pneumonic pasteurellosis might not be the best way to confirm the influences of the disease.

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