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MOLECULAR DETECTION OF AVIAN *Gyrovirus* TYPE 2 (AGV2) IN SPECIFIC PATHOGEN FREE (SPF) CHICKENS HOUSED ON COMMERCIAL BROILER LITTER

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ABSTRACT

In order to understand some aspects of biology and infectious potential capacity to AGV2 (*Circoviridae* family, *Gyrovirus* genus), 132 SPF chicks were used in the present study. Chicks were separated in two groups, one (G1) with 106 and other (G2) with 26 chickens. Chickens from G1 were maintained in eight isolators chambers units with filtered air and positive pressure, while the birds from G2 were housed in an experimental facility, reared on reused treated commercial broiler litter. To confirm that all birds from G1 were free from AGV2, feather samples (n=106) were collected and tested on the eighth day of the experiment. Subsequently, on day 20, 43 and 11 feathers of birds from G1 and G2 respectively were collected. From day 20 to day 35, on the G1, only two birds were maintained in the isolator chamber. On the final experimental period (35 day) all birds (G1 and G2) were euthanized and the feathers were tested by PCR for AGV2 DNA detection. In G1 group, all tested birds were free of AGV2 DNA by day 8 (106/106) and day 20 (43/106) of the experiment, as well as the two remaining birds until day 35 (2/2). In G2, only one chicken (1/11) had positive PCR for AGV2 DNA at day 20, while at day 35 all chickens from this group (26/26) tested positive for AGV2 DNA. The use of sentinel chickens (SPF) exposed to an environmental challenge (commercial poultry litter), proved the infectivity of the AGV2 virus even without viral isolation.

KEYWORDS: AGV2; Infectivity, Chicken; Broiler Litter

INTRODUCTION

Avian *Gyrovirus* Type 2 (AGV2) has been recently identified and suggested to belong to the *Circoviridae* family, genus *Gyrovirus* along with Chicken Anemia Virus –CAV (Rijsewijk *et al*., 2011). The CAV, a non-enveloped DNA virus, is highly resistant to chemical and heat inactivation, which gives it the ability to persist and spread in the environment (Shat and Santen, 2008). The characteristics of resistance and/or sensitivity of AGV2 to different environmental conditions are unknown. Although there is lack of studies on the prevalence of AGV2, apparently the virus is fairly widespread in different chicken populations, including backyard and commercial poultry in different geographical regions (Santos *et al*., 2012). Considering its wide distribution in chickens populations and their different tissues, and yet, its similarity to the *Gyrovirus* genus, it is speculated that its persistence in the environment could be similar to other *Gyrovirus* as CAV.

The molecular detection of AGV2 has been demonstrated in various tissues indicating no preference for specific systems or organs. The first findings related to the virus were obtained from the detection of viral DNA fragment in sera and feathers from commercial and backyard chickens (Rijsewijk *et al*., 2011; Santos *et al*, 2012). Subsequently, the viral DNA has been detected in bursa, liver, spleen, bone marrow, proventriculus, intestine, and brain (unpublished data).

The epidemiology of the virus, and pathogenesis of AGV2 including source and routes of infection and transmission, as well as the interaction agent-host-environment in chickens are not known. The knowledge about the ecology of the agent, i.e., its ability to survive and interact under different environmental conditions in poultry production figures as important aspects to elucidate the dynamics of this virus.

The poultry litter is recognized as a favorable environment to hosting and maintain bacterial, parasitic and viral agents (Silva *et al*., 2011). The reuse of poultry litter for consecutive batches of chickens is a

common practice in the Brazilian poultry industry. However, this practice requires litter treatment between batches of chickens to reduce or eliminate the risk of transfer of pathogens, to ensure adequate sanitary conditions for production of the subsequent batches. Fermentation of the poultry litter is widely used in Brazilian poultry production due effective results in the reduction of undesirable microbial agents (Silva *et al*., 2007). The objective of this study was to investigate the infectivity of AGV2 in SPFs exposed to reused commercial poultry litter previously treated by fermentation.

MATERIALS AND METHODS

One hundred and thirty two Specific Pathogen Free (SPF) chickens were used in the present experiment. The experimental procedures involving the animal utilization was evaluated by a local Animal Ethic Committee. Chickens were separated in two groups, one (G1) with 106 and other (G2) with 26 chickens. Chickens from G1 were maintained in eight isolators chambers units with filtered air and positive pressure, while the birds from G2 were housed in an experimental facility in contact with the reused treated commercial broiler litter.

To confirm that SPF chickens were free from AGV2, feather from all birds from G1 group were collected (n=106) at day 8. Subsequently, on day 20, feathers from 43 birds from G1 and 11 birds from G2 were collected. On day 20, 104 birds from G1 group were euthanized and only two birds were maintained in the isolator chamber until day 35. At the end of the study (day 35) the only two remaining birds from G1 and all birds from G2 group were euthanized and the feathers collected were tested for AGV2 using PCR. DNA extraction was performed as previously described (Bello *et al*., 2001; Davidson *et al*., 2008) with some modifications. Next, an RNase digestion was performed. Samples were stored at -20 ºC until be used. The AGV2 PCR was performed essentially as described by Santos *et al*., 2012, briefly as follow: the primers (PF: 5'-CGTGTCCGCCAGCAGAAAC-3' and PR: 5'- GGTAGAAGCCAAAGCGTCCAC-3') amplified a fragment of 345 bp and target a genomic region that codes part of the VP2 and VP3 genes (nt 656 to nt 1001), the specific products were analyzed on a 1% agarose gel.

RESULTS AND DISCUSSION

In G1 group, all tested birds were free of AGV2 DNA by day 8 (106/106) and day 20 (43/106) of the experiment, as well as the two remaining birds until day 35 (2/2). Because all chickens tested negative until day 20, in order to reduce costs, only two birds were maintained in the isolator chambers. In G2 group, only one chicken (1/11) had positive PCR for AGV2 DNA at day 20, while at day 35 all chickens from this group (26/26) tested positive for AGV2 DNA. Considering that chickens from group G1 remained free of AGV2 DNA and all chickens from G2 group became positive for AGV2 DNA at the end of the experiment, these results certainly indicates environmental infection of AGV2 in chickens from G2 group reared in reused commercial poultry litter. So far, these data has not been demonstrated until now.

The detection of AGV2 DNA in chickens of G2 group from day 20, that were previously free from the DNA of this virus, suggest that AGV2 is a complete, active and infective viral particle having the chicken as a host. The use of sentinel chickens (SPF) exposed to an environmental challenge (commercial poultry litter), proved the infectivity of the AGV2 virus, being possible the experimental reproduction of the infection even without viral isolation. Since, so far, was not possible to isolate the AGV2, the experimental reproduction of infection was not demonstrated until this moment. Given that the AGV2 appears to be widely distributed in the chickens, according suggested by Santos *et al* (2012), the reused broiler litter is a good alternative to challenge and reproduce the AGV2 infection for subsequent studies. The results event suggests that the AGV2 is resistant to fermentative treatment applied in the litter used in this experiment.

Furthermore, as described by others, the utilization of feathers showed to be a very efficient material for diagnosis, which enables the monitoring of the same bird over time without implying in invasive sampling methods (Davidson, 2009).

CONCLUSION

In the present work was reported by the first time the infection of chickens from contaminated poultry litter. It suggests that AGV2 is hat AGV2 is a complete, active and infective viral particle resistant to fermentative treatment applied. In the other hand, is important to note that feathers are very useful specimen to diagnosis and monitoring AGV2, enabling to monitor of the same bird over time without implying invasive methods.

Others research activities are in development by Embrapa Swine and Poultry, UFRGS and Central Veterinary Institute in order to achieve a better understanding about the significance of the presence of AGV2 in poultry.

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