Rapid detection of gene F of avian Metapneumovirus

B.M. Santos*; M. P. Moraes; P. R. Bárrios; L. J. Pena, S. Y. Marin

***Federal University Federal of Vicosa, 36570-000 - Vicosa, Minas Gerais, Brazil, e-mail: bmsantos@ufv.br**

In the present study, we evaluated the viability of the reverse transcription-polymerase chain reation (PCR) in the detection of gene F of the avian Metapneumovirus. An isolate of avian Metapneumovirus from chickens with swollen head syndrome was propagated in Vero cells. After obtaining cytophatic effect, the cells were detached from the surface and centrifuged. Total RNA was extracted from infected cell by Trizol method. A par of oligonucleotide primers corresponding to sequence of the F protein was used to prepare cDNA. The sequences for the primers were 5'-AAAGTCAGCACAGGTAGACAC-3' and 5'-AAATAACTTAACTGACATAAGCCAT-3' of the fusion protein (F) of the mRNA. The reaction was conduced in the conditions following: denaturation in 94C by 30s; annealing in 54C by 30s; and extension, 72C by 60s. For the visualization of amplified PCR products, were used the ethidiun bromide staining after electrophoresis in 1% agarose gel and photographed under ultraviolet light. The results obtained showed that the Trizol method sustained the RNA complete to the cDNA synthesis. In the cDNA amplification reaction, the primers showed to be extremely specific to detection of the virus genome. It follows that the RT-PCR is a useful tool for the rapid detection of Avian Metapneumovirus.

Key words: Avian Metapneumovirus, reverse transcription-polimerase chain reaction, and swollen head syndrome.

Introduction

The avian Metapneumovirus is the etiologic agent of the swollen head syndrome (SHS) in poultry and rinotracheitis in turkey. The virus causes acute respiratory tract infection both in turkeys and chickens with sudden and rapid spread through flocks. This virus is responsible for significant losses in broilers where it occurs, and the losses attain 05-30%. Outbreaks of SHS in chickens are preceded by immunosupression, and require exposure to an avian Metapneumovirus as the primary viral agent, and subsequent secondary infection with *Escherichia coli*. The isolation of the avian Metapneumovirus of birds, with the clinical disease, is very arduous (Santos et al., 2005). The diagnostic is usually based on serology using ELISAs, but the available kits give variable results, interpretation is difficult and improved diagnostic tests are required (Lima et al., 1998). The polymerase chain reaction (PCR) is a new diagnostic technique, which is used to diagnostic in many infectious diseases. In this technique is amplificated the target region of the DNA of the infectious agent (Reubel, et al., 1998). In the present study, we evaluated the viability of the reverse transcription-polymerase chain retain (RT-PCR) in the detection of gene F of the avian Metapneumovirus.

Materials and methods

Was used an avian Metapneumovirus A, which was isolated of chickens flocks with SHS. The virus samples was propagated in Vero cell culture. This material was centrifuged at 10,000 x g for 10 min at 4 C, and the supernatant was used for isolation of viral RNA. The viral isolated was with Trizol Reagent by the manufacture's protocol. The RNA was eluted in 50 μl of diethylpyrocarbonate (DEPC)-treated water. The isolated RNA was stored at –20 C for later use.

A par of oligonucleotide primers corresponding to sequence of the F protein was used to prepare cDNA. The sequences for the primers were 5'-AAAGTCAGCACAGGTAGACAC-3' and 5'-AAATAACTTAACTGACATAAGCCAT-3' of the fusion protein (F) of the mRNA (Jing et al., 1993).

The RT-PCR was performed with the Thermoscript II RT-PCR kit (Life Technologies, Inc.). Five microliters of viral RNA was used for cDNA synthesis. The reaction was incubated at 70 C for 10 min and then at 42 C for 5 min. Heating at 70 C for 15 min terminated the reaction. In the PCR retain were used 0,2 ml of cDNA and 0,5 ml of forward and reverse primers. The mixture was denatured at 94 C by 30s, annealing at 54 C by 30s, and extension at 72 C by 60s. The resulting products were subjected to electrophoresis with a 1% agarose gel. The gel was stained with ethidium bromide and visualized with an ultraviolet transilluminator and photographed under ultraviolet light..

Results and Discussion

The results obtained showed that the Trizol method sustained the RNA complete to the cDNA synthesis. The cDNA was synthetized with the used of reverse transcriptase (RT). This enzyme transcribed the RNA in cDNA that was amplified. For the visualization of amplified PCR products, were used the ethidiun bromide staining after electrophoresis in 1% agarose gel and photographed under ultraviolet light.

The primers designed from the sequence of the F gene for avian Metapneumovirus, 5'-AAAGTCAGCACAGGTAGACAC-3' and 5'-AAATAACTTAACTGACATAAGCCAT-3', were specific to detect a product with 541 pb (Fig. 1).

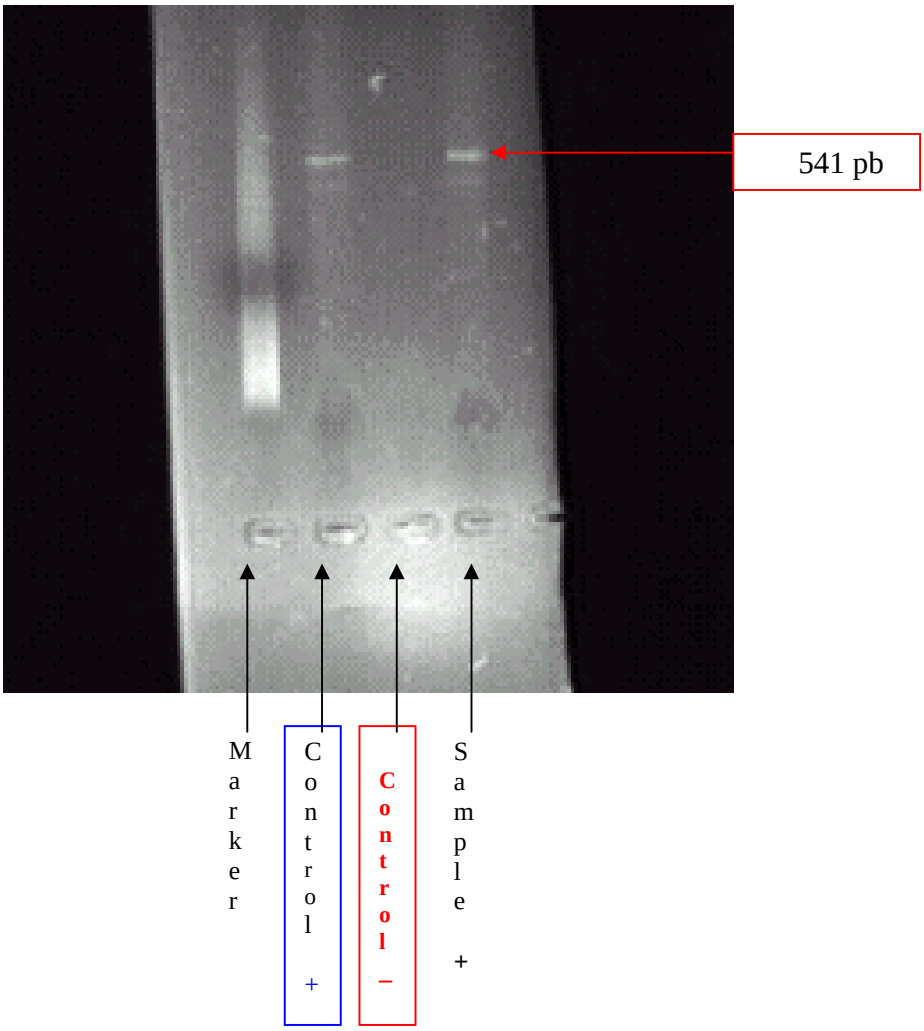

Figure 1. Ethidium bromide-stained agarose gel after eletrophoresis of RT-PCR products. Of left to right: (1) molecular weight marker (Hind III); (2) positive control; (3) negative control; and (4) products obtained with the primer-pair, with 541pb.

In this study confirm that the RT-PCR tecnic is fast and it showed very sensibility. The technique is capable to replace the laborious technique to isolate and identification virus.

A par of oligonucleotide primers corresponding to sequence of the F protein, which was used to prepare cDNA are specific to detect the avian Metapneumovirus. The specificity of the technique depend only of the knowledge of the sequence of the target region (Tiwari et al., 2003).

Conclusion

In the cDNA amplification reaction, the primers showed to be extremely specific to detection of the virus genome. It follows that the RT-PCR is a useful tool for the rapid detection of Avian Metapneumovirus.

References

HAFEZ, H.M. and HESS, M. (1999) Modern tecniques in diagnosis of poultry diseases: Review. *Arch. Geflügelk* **63**:237- 245.

JING, L., COOK, J.K.A., and BROWN, T.D.K. (1993) Detection of turkey rhinotracheitis virus in turkeys using the polymerase chain restion. *Avian Pathology* **22**: 771- 783.

LIMA, A.M.C., SANTOS, B.M., PATARROYO, J.H.S. (1998) Brasilian Jornal of Poultry Science. **5**:.27-35.

REUBEL, G.H. AND STUDDERT, M.J. (1998) Benefits and limitations of polymerase chain reation (PCR) in veterinary diagnostic virology. *Veterinary Bulletin* **68**: 505-516.

SANTOS, B.M., FARIA, J.E., RIBEIRO, V.V. (2005) *Doenças virais de importância nas aves*. **n. 13**, pp 65-67. Vicosa: UFV.

TIWARI, A.K., KATARIA, R.S. and PRASAD, N. (2003) Differectiation of infectious bursal disease viruses by restrction enzime analysis of RT-PCR amplified VPI gene sequense. Comparative Immunology and Microbiology Infectious Diseases **26**: 47-53.