International Scholars Journals

African Journal of Virology Research ISSN 3421-7347 Vol. 13 (7), pp. 001-003, July, 2019. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Short Communication

Use of virus suspensions without RNA extraction as RT-PCR templates for detection of Newcastle disease virus

P. N. Wambura*

School of Veterinary Science, University of Queensland, Brisbane, QLD 4072, Australia. E-mail: phil_wambura@yahoo.com, pwambura@suanet.ac.tz.

Accepted 12 April, 2019

Allantoic fluid (AF) and cell culture supernatant (CCS) obtained from eggs or cells infected with strain I-2 of *Newcastle disease virus* were processed by different RNA template preparation methods for direct use in reverse transcriptase-polymerase chain reaction (RT-PCR). The objective was to determine the most effective technique for viral RNA extraction with consideration for efficacy, economy and simplicity. Results showed that use of undiluted CCS without RNA extraction or other treatment as template for RT -PCR produced a positive signal whereas direct use of undiluted AF did not. When aliquots of each sample dilution were used, an amplicon was detected from 1:10 dilution of both AF and CCS whereas no PCR products were amplified from both AF and CCS at 1:100 dilution. Both boiled undiluted AF and CCS produced positive signals when were used as templates for RT- PCR. An important contribution of the present study is the evidence that crude CCS, diluted or boiled AF and CCS may be used directly in RT -PCR without further manipulation, and yielded a positive PCR result comparable to those obtained from RNA extracted by silica gel based method.

Key words: Newcastle diseases virus, polymerase chain reaction, RNA treatment, strain I-2.

INTRODUCTION

The development of reverse transcriptase-polymerase chain reactions (RT-PCR) has revolutionalised the detection of RNA viruses in tissues and body fluids. It is now possible to detect RNA viruses at a high level of sensitivity in infected materials. Jestin and Jestin (1991) were the first to detect *Newcastle disease virus* (NDV) in infective allantoic fluids using PCR. Since then several RT-PCR methods have been developed and applied in molecular studies of NDV (Belak and Ballagi-Pordany, 1993; Kant et al., 1997; Cavanagh 2001; Wang et al., 2001; Aldous and Alexander 2001).

The extraction and purification of RNA is an important initial step in RT-PCR for successful detection of infectious agents by this technique. No studies have been done on the comparison of RNA extraction methods from cell culture and allantoic fluid containing NDV. The objective of the present study was to compare and to evaluate a simple, fast, cheap, reliable and effective method for treatment of RNA from NDV infective materials.

MATERIALS AND METHODS

Virus

The strain I-2 of Newcastle disease (ND) vaccine was propagated in 10-day-old embryonated chicken eggs from a working stock that had been produced by one passage from the vaccine master seed, as described by Spradbrow et al. (1995) and Alexander (1998). Non-infective allantoic fluid was used as a negative control. All eggs used in this study were obtained from a reputable commercial hatchery and poultry-breeding farm in Brisbane. The chickens on this farm were not vaccinated and were free from ND. Preparation and propagation of I-2 virus in chick embryo fibroblast (CEF) cells was done as described by Uruakpa (1997). The 50% tissue culture infectious doses ($TCID_{50}$) and 50% embryo infectious doses (EID_{50}) were

***Present address:** Sokoine University of Agriculture, Department of Veterinary Microbiology and Parasitology, P. O. Box 3019, Chuo Kikuu, Morogoro, Tanzania. Tel (Landline): 255 23 460 4557. Tel (mobile): 255 744 638 460. Fax: 255 23 2604647.

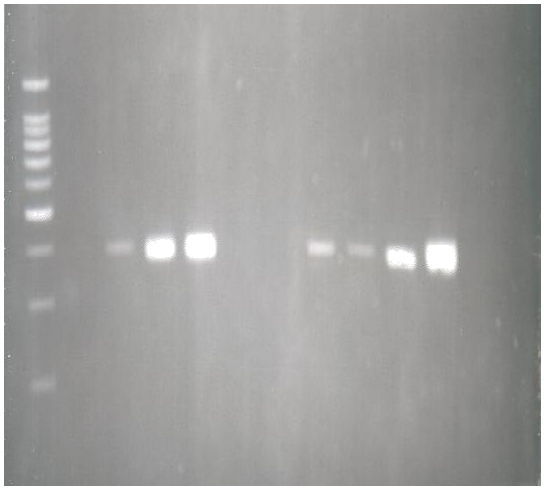

Figure 1. Electrophoretic profiles of PCR products from different viral RNA template preparations. M = DNA ladder (100 bp), lane 1 = Direct allantoic fluid (AF), lane 2 = Direct Chicken embryo fibroblast (CEF), lane 3 = Diluted AF 1:10, lane 4 = Diluted CEF 1: 10, lane 5 = Diluted AF 1:100, lane 6 = Diluted CEF 1:100, lane 7 = Direct use undiluted boiled AF, lane 8 = Direct use undiluted boiled CEF, lane 9 = Spin and silica gel AF, lane 10 = Spin and silica gel CEF, lane 11 = Negative sample AF, lane 12 = Negative sample CEF.

calculated as described by Reed and Muench (1938).

Preparation of templates for RT-PCR

Infective and non-infective allantoic fluid (AF) and CEF cell culture supernatant (CCS) were subjected to different treatments before use in RT-PCR:

Direct use of undiluted samples- 5 µL of either AF or CCS was subjected to RT-PCR with no further manipulation.
Direct use of diluted samples-AF or CCS were diluted 1:10 and 1:100 in distilled water, and from each aliquot 5 µL was added into PCR tubes and subjected to RT-PCR with no further manipulation.
Direct use of boiled samples-AF or CCS were boiled for 3 min and immediately cooled on ice. Thereafter 5 µL of the aliquots were added into PCR tube ready for RT-PCR.

Silica gel based spin column method (QIAamp® viral RNA kit, Qiagen). The kit was used according to the manufacturer's instructions. Briefly, a total of 140 µL of each clarified AF or CCS were incubated with AVL buffer for 10 minutes. After viral particle lysis, the samples were mixed with ethanol and applied to the spin column provided, centrifuged and washed twice with buffers AW1 and AW2. RNA was then eluted from columns using 60 µL of AVE buffer, and stored at –20°C until further use. 5 µL of this aliquot was used in RT-PCR.

Primers

Two degenerate primers, forward: 5′-GTYAAYATATACACCTC ATCYCAGACWGG-3′(Y=C or T, W=A or T) and reverse: 5′-CTGCCACTGCTAGTTGBGATAATCC-3′ (B=G, C or T) which recognize sequences surrounding the cleavage site for the NDV F_0 protein (Jorgensen et al., 1999) were used in this study. GeneWorks Pty Ltd (SA) synthesized the oligonucleotide primers.

Single tube RT-PCR

The RT-PCR reaction was carried out according to the manufacturer's instructions for the Access RT-PCR system Kit (Promega, prod. no A1250). The amplification was carried out in a thermocycler (PCR Sprint, Hybaid Ltd). The thermocycling profile for single tube RT-PCR included: cycle 1: 48°C for 45 min (RT reaction); cycle 2: 95°C for 2 min; cycles 3-42 (40 cycles): 94°C for 30 s, 60°C for 1 min, 68°C for 2 min and cycle 43: 68°C for 7 min (final extension). The amplified products were analysed on 1.5% agarose gel. The experiments above were repeated three times.
The RNA was amplified from the virus from AF and CCS with initial titres of $1x\ 10^4$ EID_{50}/mL and $1x10^5$ $TCID_{50}$/mL, respectively.

RESULTS

The results of comparative evaluation of different RNA template preparation methods from AF and CCS infected with strain I- 2 of NDV showed that using CCS directly as template for RT-PCR produced a positive signal whereas direct use of undiluted AF did not. However, when aliquots of diluted AF or CCS were used directly as templates, an amplicon was detected using 1:10 dilution of both AF and CCS, whereas no PCR products were amplified from either AF or CCS at 1:100 dilution. Use of boiled, undiluted AF and CCS as templates for RT-PCR both produced positive signals (Figure 1).
All samples from non-infective AF or CCS used directly or processed by RNA extraction technique produced negative RT-PCR results. Moreover, as shown in Figure 1, very strong bands were produced when RNA extracted from infective AF or CCS by silica gel based spin column method was used as template for RT-PCR.

DISCUSSION

Because of the large number of samples normally required in molecular epidemiological studies, it is also desirable that the method employed for testing be simplified as much as possible. Current testing procedures typically require RNA extraction and purification from target samples before used in RT-PCR
(Belak and Ballagi-Pordany, 1993). RNA extraction step can be quite laborious and time consuming.

An important contribution of the present study is the evidence that crude CCS, diluted or boiled AF and CCS could be used directly in RT-PCR without further manipulation and could yield positive results, which were comparable to the bands obtained from RNA extracted by Silica gel based method. Crude CCS as template for RT-

PCR produced electrophoretic bands, suggesting that no PCR inhibitors were present in the fluid, which was not the case when AF was used. However, when AF was diluted (1:10) or boiled (undiluted) and used as template for RT-PCR, a positive signal was detected, indicating that these treatments inactivated or minimized the effect of the inhibitors in AF. Further dilution of AF resulted in negative results. This may be due to the fact that there were not enough RNA templates to be amplified by RT-PCR.

The use of crude samples in this study resulted in fast, cheap, reproducible and effective methods for provision of RNA templates for RT-PCR. Removal of the RNA extraction step reduced sample handling time and the potential for sample cross-contamination.

The QIAamp viral RNA method represents a technology for extraction of RNA that combines selective binding properties of a silica gel based membrane with the speed of microspin technology. It produces total RNA of the high quality and quantity but it is the most expensive method among other RNA extraction methods. This is in agreement with previous studies by (Xiang et al., 2001) which found Qiagen kit to be a very efficient but expensive method.

In the present study, AF and CCS infected with strain I-2 of NDV were either used directly (undiluted and diluted) or after RNA treatment for use in RT-PCR to determine which is the most effective technique for viral RNA processing, with consideration for efficacy, economy and simplicity. All other steps of RT -PCR were kept constant. In this study, it was shown that good amplification results could be achieved even from crude samples.

ACKNOWLEDGEMENTS

The study was undertaken by the author while under the scholarship through Tanzania Agricultural Research Project Phase II funded by the World Bank.

REFERENCES

Aldous E W, Alexander D J (2001). Detection and differentiation of *Newcastle disease virus* (avian paramyxovirus type 1). Avian Pathol. 30: 117-128.

Alexander D J (1998). Newcastle disease. In: Swayne D E, Glisson J R, Jackwood M W, Pearson J E, Reed W M (eds) A laboratory manual for isolation and identification of avian pathogens. American Association of Avian Pathologists, Kennett Square, PA, pp156-163.

Belak S, Ballagi-Pordany A (1993). Application of the polymerase chain reaction (PCR) in veterinary diagnostic virology. Vet. Res. Comm. 17: 55-72.

Cavanagh D (2001). Innovation and discovery: The application of nucleic acid-based technology to avian virus detection and characterization. Avian Pathol. 30: 581-598.

Jestin V, Jestin A (1991). Detection of Newcastle disease *virus* RNA in infected allantoic fluid by in vitro enzymatic amplification (PCR). Arch. Virol. 118: 151-161.

Jorgensen H, Jensen Handberg K, Ahrens P, Hansen H C, Manvell R J, ALexander D J (1999). An outbreak of Newcastle disease in free-living Pheasants (Phasianus colchicus). J. Vet. Med. Series B. 46: 381-387.

Kant A, Roch D J, Roozelaar V, Balk F, Huurne A T, (1997). Differentiation of virulent and non-virulent stains of Newcastle disease virus within 24 hours by polymerase chain reaction. Avian Pathol. 26: 837-849.

Reed L S, Muench L H (1938). A simple method of estimating fifty percent endpoints. Am. J. Hyg. 27: 493-497.

Spradbrow P B, Mackenzie M, Grimes S E (1995). Recent isolates of Newcastle disease virus. Vet. Microbiol. 46: 21-28.

Uruakpa U A (1997). Preservation of Newcastle Disease vaccine at room temperature. M.V.Sc. thesis, University of Queensland, Australia.

Wang Z, Vreede F T, Mitchell J O, Viljoen G J (2001). Rapid detection of *Newcastle disease virus* isolates by a triple one-step RT-PCR. Onderstepoort J. Vet. Res. 68: 131-134.

Xiang X, Qiu D, Hegele R D, Tan W C (2001). Comparison of different methods of total RNA extraction for viral detection in sputum. J. Virol. Methods. 94: 129-135.