

Propagation of bovine parainfluenza type 3 virus in chicken embryos

A. S. Al-Bana and A. B. Allawe

College of Veterinary Medicine\ University of Baghdad

Abstract

Bovine parainfluenza type 3 virus (BPIV-3) was first isolated in secondary embryonic bovine kidney cell culture (SEBK), and then the virus was propagated for the first time in chicken embryos by allantoic inoculation method. The virus was propagated for four passages in chicken embryos, then the propagated virus was inoculated in SEBK cell culture and cytopathic effects of inoculated virus (focal rounding of cells with syncytia) were noticed. Hemagglutination test was used to detect the virus in harvested allantoic fluid with 0.5% guinea pig RBCs. The virus was diagnosed by hemagglutination inhibition test with 0.5% guinea pig RBCs by using standard antiserum for BPIV-3. Reverse-transcriptase polymerase chain reaction (RT-PCR) was used for detection of propagated BPIV-3 in chicken embryos allantoic fluids.

تنمية فايروس نظير الأنفلونزا البقري نوع 3 في اجنة البيض النامي

أنطوان صبري البنا وعائده برع علاوي

كلية الطب البيطري/ جامعة بغداد

الخلاصة

تم تنمية فايروس نظير الأنفلونزا البقري نوع 3 المعزول في الخلايا الثانوية لجنين البقر ولأول مره في العراق في اجنة البيض النامي عن طريق الحقن في كيس الالنتوي، إذ تم تمرير الفايروس اربع مرات في اجنة البيض النامي تم بعد ذلك اعادة تنمية الفايروس في الخلايا الثانوية لجنين البقر، إذ لوحظت التأثيرات المرضية الناتجة عن نمو الفايروس في الخلايا كأستدارة الخلايا مع تكوين الخلايا العملاقة. استخدم اختبار التلازن الدموي لتشخيص الفايروس في سائل الالنتوي بأستخدام خلايا الدم الحمراء لخنزير غينيا بنسبة 0.5% كما استخدم اختبار اثباط التلازن الدموي لتشخيص الفايروس بأستخدام مصل قياسي مضاد لفايروس نظير الأنفلونزا البقري نوع 3 بأستخدام خلايا الدم الحمراء لخنزير غينيا بنسبة 0.5%. استخدم فحص تفاعل سلسلة البوليمريز لتشخيص الفايروس في سائل الالنتوي المحصود لخلايا اجنة البيض النامي.

Introduction

Bovine parainfluenza type 3 virus (BPIV-3) is a long recognized currently under appreciated endemic infection in cattle populations (1), clinical disease is most common in calves with poor passive transfer or decayed maternal antibodies (2). It is usually mild consisting of fever, nasal discharge and dry cough (3), caused at least partly by local immunosuppressive effect, BPIV-3 infection is often complicated by coinfection with other respiratory viruses and bacteria and is therefore an important component of enzootic pneumonia in calves and bovine respiratory disease complex in feedlot cattle(4). Bovine parainfluenza type 3 virus (BPIV-3) was first isolated in tissue culture of embryonic bovine kidney cell culture (SEBK) in 1959 (5). In Iraq the virus was first isolated in tissue culture of embryonic bovine kidney cell culture (SEBK) in 2011 (6). Many reports indicated that BPIV-3 can be isolated and produced in 14-day-old embryonated chicken eggs and that the developing chick embryo is highly susceptible to parainfluenza -3 virus infection (7). This study indicates that BPIV-3 can be propagated in 11 day old embryonated chicken eggs by allantoic inoculation method.

Materials and Methods

- **Chicken embryos: 11-day old chicken embryos (10 chicken embryos) were used for BPIV-3 propagation** by allantoic inoculation method.
- **Guinea pig RBCs:** 0.5% of guinea pig RBCs in normal buffer were used for heamagglutination and heamagglutination inhibition tests.
- **Reference antiserum:** Standard Bovine PI3 antiserum from ELISA kit (Cypress Diagnostics/Belgium).
- **Secondary embryonic bovine kidney (SEBK) cell culture** was prepared as described by Baron, *et al.*, (1995) (8).
- **Extraction kit:** The kit was used for RNA extraction of viral RNA (from isolated virus) as instruction of manufacturer Company (Primer Design Ltd Precision TM Viral RNA/DNA extraction kit Handbook HB11.02.01).
- **BPIV-3 Amplification PCR kit (Ref. K131)-Manual two steps:** The amplification kit has been manufactured by Genekam Biotechnology AG, Germany to detect BPIV-3.

Methods:

- **Virus inoculation:** Bovine parainfluenza virus type 3 was grown in SEBK cell culture (seventh passage) was inoculated in chicken embryos by allantoic inoculation method 0.2 ml of harvested infected SEBK cell culture was inoculated in each 10 chicken embryos and 5 chicken embryos were inoculated by mutinous MEM media as a control group. The chicken embryos were examined daily by candling method. After five days of virus inoculation, the allantoic fluid was harvested and heamagglutination activity was detected in allantoic fluid and then heamagglutination inhibition test was performed. This process was repeated four times in chicken embryos by allantoic inoculation method.
- **SEBK cell culture inoculation:** after 5 days at 37c, the passage four of allantoic fluids were harvested and tested for virus by inoculation of SEBK cell culture.
- **Heamagglutination method:** Micro method was performed by using 0.5% guinea pig RBCs (9, 10).
- **Heamagglutination inhibition test: B- micro method** was performed by using 0.5% guinea pig RBCs, standard antiserum for BPIV-3 and 4 HA units of propagated virus in chicken embryos (allantoic fluid) (9, 10).
- **RNA isolation, cDNA synthesis and PCR amplification:** Total viral RNA was extracted from viral stocks with primer design kit according to manufacturer's instructions. cDNA synthesis of the genomic RNA and PCR amplification were performed with amplification PCR kit (Ref. K131).

Results

- **Virus inoculation:** The propagated virus didn't kill chicken embryos in four passages after five days of virus inoculation. Hemorrhage was seen on infected embryos as compared to non infected embryos (Fig. 1).



Fig. (1) Hemorrhage and dwarfing of infected embryo as compared with non infected embryo

- **SEBK cell culture inoculation:** The cytopathic effects were noticed after 3 days post virus inoculation (allantoic fluid) in SEBK cell culture (first passage), focal rounding of cells with giant cells formation, on the subsequent passage the cytopathic effects were noticed after 2 days with focal rounding of cells and giant cell formation as seen in (Fig. 2, 3) in compare with non inoculated SEBK cell culture as seen in (Fig. 4).

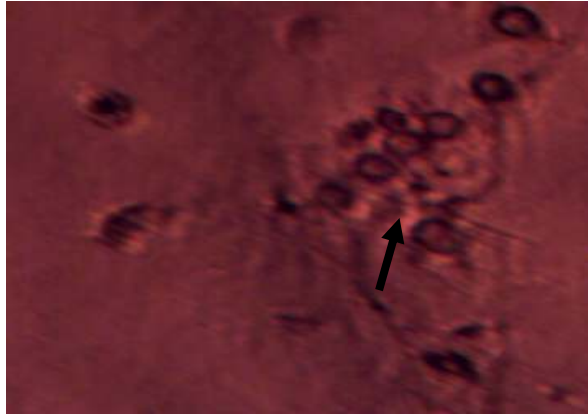


Fig. (2) SEBK cell culture after 5 days of virus inoculation, focal rounding of cells with syncytia (→) 200x



Fig. (3) SEBK cell culture after 5 days of virus inoculation, focal rounding of cells with syncytia (→) 100x.



Fig. (4) Normal non inoculated SEBK cell culture 200x

- **Heamagglutination test:** The results of hemagglutination showed ability of isolated virus to agglutinate guinea pig RBCs it indicated positive result started with titer 4 HA unit in the first passage to 32 HA unit in the fourth passage; titer was calculated according to (11) as explained in Table (1).

Passage number in chicken embryos	Heamagglutination Unit/0.5%g.pig RBCs
1-First passage	4 HA
2-Second passage	4 HA
3-Third passage	16HA
4-Fourth passage	32HA

- **Heamagglutination inhibition test:** The standard positive serum for BPIV-3 gave positive result to inhibit the heamagglutination of 4 HA units of the propagated virus while the titer of the standard positive serum was 16 HA units.
- **Reverse transcriptase-polymerase chain reaction (RT-PCR):** The reverse transcriptase- polymerase chain reaction (RT-PCR), using primers representing part of BPIV-3 heamagglutinin- neuraminidase gene (HN), amplified a fragment of approximately 119 base pair (bp) with material extracted from allantoic fluid of infected chicken embryos (fourth passage) (Fig. 5).

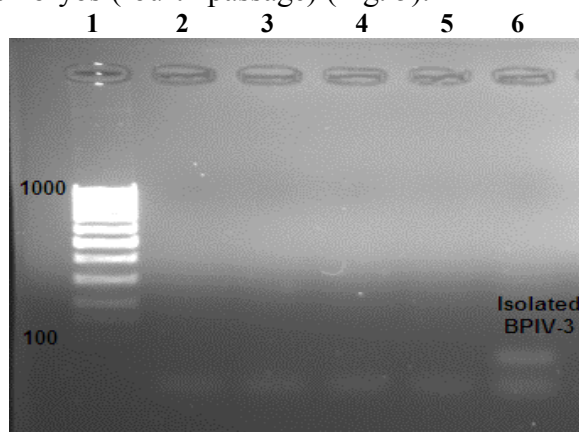


Fig. (5) RT-PCR amplification. Results showing 119 bp fragments amplified from fourth passage of isolated BPIV-3(grown in chicken embryos) in IRAQ. Products were analyzed in 2% agarose gel electrophoresis. Lane 1: molecular size marker (1000-100) bp; lane 2: negative control; lane 3, 4 and 5: first, second and third passage of isolated BPIV-3 (grown in chicken embryos); lane 6: fourth passage of isolated BPIV-3(grown in chicken embryos).

Discussion

Bovine parainfluenza type 3 virus is the most common virus infection of respiratory tract of cattle(12). Active infection can be diagnosed by virus isolation from nasal swabs or immunofluorescence testing on smears made from nasal swabs (13). The isolation of BPIV-3 in SEBK cell culture can be complicated by presence of infectious bovine rhinotracheitis (IBR) which also produces cytopathic effects in SEBK cell culture(14), but IBR do not interfere with the isolation of BPIV-3 in chicken embryos (7), Therefore many attempts were made to isolate BPIV-3 in chicken embryos (7). Based on results were obtained in this study, BPIV-3 can be grown in chicken embryos by allantoic method this agreed with others (7) and the virus did not kill embryos and this agreed with (15) who explained that the infection with BPIV-3 is mild unless complicated with viral and bacterial infection. Cytopathic effects of Propagated virus (in chicken embryos) in SEBK cell culture were focal rounding of cells with syncytia due to presence of fusion protein in virus envelope(16). Heamagglutination test was positive to detect BPIV-3 in allantoic fluid due presence of heamagglutinin glycoprotein in the

envelope of virus (17). Hemagglutination inhibition test was positive to detect BPIV-3 in allantoic fluid by using standard positive serum for BPIV-3. Recently, amplification of gene HN fragment was utilized for identification and strain differentiation of bovine parainfluenza-3 virus in Northern American viral strains and Russian isolates (18). The RT-PCR described here, using primers representing part of BPIV-3 hemagglutinin-neuraminidase gene (HN), amplified a fragment of approximately 119 bp with material extracted from infected allantoic fluid. Within the conditions employed in this study the specificity of the RT-PCR was high in agreement with other studies (13). The RT-PCR presented in this study is adequate for detection of BPIV-3 from infected allantoic fluid for the first time in agreement with other studies in cell cultures (13).

References

1. Fulton, R. W.; Purdy, C. W.; Confer, A. W.; Saliki, J. T.; Loan, R. W.; Briggs, R. E. & Burge L. J. 2000. Bovine viral diarrhea viral infections in feeder calves with respiratory disease: interactions with *Pasteurella* spp., parainfluenza-3 virus, and bovine respiratory syncytial virus. *Can. J. Vet. Res.*, 64:151-159.
2. Bryson, D. G. 1990. Parainfluenza-3 virus in cattle. In: *Virus infections in Ruminants* (Eds. Dinter, Z. & Morein, B.). Elsevier. Amsterdam, PP. 319-333.
3. Cusack, P. M.; McMeniman, N. & Lean, I. J. 2003. The medicine and epidemiology of bovine respiratory disease in feedlots. *Australian Vet. J.*, 81:480-487.
4. Loneragan, G. H.; Gouldm, D. H. M.; Mason, F. B.; Garry, G. S. & Mills, L. J. 2001. Involvement of microbial respiratory pathogens in acute interstitial pneumonia in feedlot cattle. *AJVR*, 62(10):1519-1524.
5. Reisinger, R. C.; Heddleston, K. L. & Manthei, C. A. 1959. A myxovirus (SF-4) associated with shipping fever of cattle. *J. Am. Vet. Med. Assoc.*, 135:147-152.
6. Allawe, A. B. & Al-Bana, A. S. 2012. Detection of bovine parainfluenza -3 in Iraq. Proceeding of 11th scientific conference of veterinary medicine/University of Baghdad.
7. Ronald, G. M. 1964. Isolation of bovine parainfluenza-3 virus in chick embryos. *J. Bacteriol.*, 88 :267-268.
8. Baron, E. J.; Peterson, L. & Finegold, S. M. 1995. Laboratory methods in basic virol. PP. 634-688. In: Baily and Scott's (ed), *Diagnostic Mycobid.*, 9th Ed.
9. Singh, K. V. & Baz, T. I. 1966. Isolation of PI-3 from water buffaloes in Egypt. *Nature Land.*, 210:616-656.
10. Jehan, A. M.; Gafer, H. A.; Hussain, A. S. & Reda, I. M. 2009. Isolation and characterization of PI3 virus from sheep and goats. *Int. J. Virol.*, 5:28-35.
11. Allan, W. H.; Lancaster, J. H. & Toth, B. 1978. Newcastle disease vaccines, their production and use. F.A.O. Rome.
12. Plummer, P. J.; Rohrbach, B. W.; Daugherty, R. A.; Daugherty, R. A.; Thomas, K. V.; Wilkes, R. P.; Duggan, F. E. & Kennedy, M. A. 2004. Effect of intranasal vaccination against bovine enteric coronavirus on the occurrence of respiratory tract disease in a commercial backgrounding feedlot. *J. Am. Vet. Med. Assoc.*, 225:726-731.
13. Vaucher, R. de A.; Simonetti, A. B. & Roehle, P. M. 2008. RT-PCR for detection of bovine parainfluenza virus type 3 (bPIV-3). *Acta Scientiae Veterinariae*. 36(3): 215-220.
14. Tjornehoj, K.; Uttenthal, A.; Viuff, B.; Larsen, L. E.; Rontved, C. & Ronsholt, L. 2003. An experimental infection model for reproduction of calf pneumonia with bovine respiratory syncytial virus (BRSV) based on one combined exposure of calves. *Res. Vet. Sci.*, 74: 55-65.
15. Hains, D. M. & Chelack, B. J. 1991. Technical considerations for developing enzyme immunohistochemical staining procedures on formalin fixed paraffin-embedded tissues for diagnostic pathology. *J. Vet. Diag. Invest.*, 3:101-112.
16. Yin, H. S.; Paterson, R. G. & Wen, X. 2005. Structure of the uncleaved ectodomain of the paramyxovirus (hPIV3) fusion protein. *Proc. Natl. Acad. Sci. U.S.A.*, 102: 9288-9293.
17. Porotto, M.; Murrell, M. & Greengard, O. 2001. Triggering of human parainfluenza virus 3 fusion protein (F) by the hemagglutinin-neuraminidase (HN) protein: an HN mutation diminishes the rate of F activation and fusion. *J. Virol.*, 77:3647-3654.
18. Vecherov, A. E.; Aianot, P. K.; Timina, A. M. & Lisitsin, V. V. 2003. Detection and differentiation of the bovine parainfluenza-3 virus strains studied by amplification and sequencing of the HN gene. *Vopr Virusol.*, 48: 46-49. (Russian).