Journal of Animal and Veterinary Advances 13 (16): 1002-1008, 2014
ISSN: 1680-5593


# Molecular Survey and Phylogenic Analysis of Infectious Bronchitis Virus (IBV) Circulating among Chicken Flocks in Riyadh Province, Saudi Arabia

[1]Yousef M. Al-Hammad, [2]Adel I. Al-Afaleq and [3]Mahmoud H.A. Mohamed
[1]Veterinary Diagnostic Laboratory, Ministry of Agriculture, 11454 Riyadh, Saudi Arabia
[2]Department of Health Environmental, College of Applied Medical Sciences,
University of Dammam, 31442 Dammam, Saudi Arabia
[3]Department of Avian Medicine, Faculty of Veterinary Medicine,
Zagazig University, 44511 Zagazig, Egypt

**Abstract:** Infectious bronchitis is a disease of an economical importance of chickens. As result of emerging of new variant strains, the prevention and control of the disease is difficult. The study is the first to be conducted in Saudi Arabia. Results showed that there is a wide-spread of IBV amongst chicken farms in the Riyadh Province. Using RT-PCR, viral RNA was detected in 35 (42.68%) out of 82 poultry farm houses from 42 farms investigated in this study. The highest viral detection (55.55%) was recorded in Shaqra governate while the lowest (20%) was in the Northern governates (AL-Zolfi, AL-Dwadmi and Hremla). Detection of IBV in vaccinated chickens was evident that some of the vaccines used in the field did not provide the adequate protection against the disease. This investigation showed that viral detection was successfully done in 29 (35.36%) out of 82 farm houses from 42 farms using the conventional method of sample collection while the detection rate dropped to 21.95% (18 farms) when Flinders Technology Associates (FTA) card were used. Interestingly enough, viral detection was found positive in 6 samples using the FTA card but was negative using the conventional method. For further identification and phylogeny, the amplified PCR products of 2 isolates were sequenced and analyzed for phylogenetic analysis and compared with other avialable IBV strains from the GenBank. First isolate was designed as IBV/CHICKEN/KSA/101/2010)with accession No. HQ916668 was found to share high nucleotide sequence identity (98.9%) for Nucleocapsid (N) protein genes with IBV/CH/LDL/01I sequence; a strain from China which may be revertant attenuated vaccine strains that have arisen as a result of the widespread use of IBV vaccines in the local poultry population in China. The second isolate was designed as IBV/CHICKENS/KSA/102/2010 with accession number HQ916669 was found to share a high degree of identity (99.7 and 99.5%) with the IBV/INDIA/TN/92/03 and IBV/HB/CH isolated in India and China, respectively. The phylogenetic analysis for the isolates were found highly diverse from the same sequences of IBV/H120 strain that commonly used as commercial vaccine for prevention of IBV in poultry flocks in KSA.

**Key words:** Prevention, vaccines, detecion, chicken, disease

## INTRODUCTION

Infectious bronchitis is one of the most economically significant diseases of intensive poultry production. The disease firstly reported in 1931 and defined as a highly contagious upper respiratory disease (Schalk and Hawn, 1931). The name of the disease refers to its most frequent clinical manifestation, although, it can infect many other epithelial cells including kidney, genital organs and many part of the alimentary tract (Cavanagh, 2005). Infectious bronchitis virus is the etiological agent of this condition and fall in genus *Coronavirus* of family Coronaviridae contains a single stranded positive sense RNA of 27.6 kb (Murphy *et al*., 1999). The genome consists of four structural proteins, namely a spike glycoprotein (S) an integral membrane glycoprotein (M), a nucleocapsid protein (N) and a small membrane envelope protein (E) (Cavanagh, 1981; Sutou *et al*., 1988). More than fifty serotypes of IBV have been identified and new variants continue to emerge despite the use of different live attenuated and/or killed IBV (Ignjatovic *et al*., 1997). It is very important to identify the field strains of IBV in circulation in order to select suitable vaccine strains for use in different geographical regions (Liu *et al*., 2006).

**Corresponding Author:** Yousef M. Al-Hammad, Laboratory of Veterinary Diagnostic, Ministry of Agriculture, 11454 Riyadh, Saudi Arabia

Traditional methods for the identification of IBV serotypes include hemaglutination-inhibition and virus neutralization tests both of these procedures are labor-intensive and time consuming. Since the early 1990s, Reverse Transcription Polymerase Chain Reaction (RT-PCR) technology has been used successfully to N protein located in the capsid of the virion is involved in RNA replication and carries group-specific antigenic determinates (Krapez *et al*., 2009). Nucleotide sequencing of *N* and *S1* genes fragment is the most useful technique for the differentiation of IBV strains and has becomes the genotyping method of choice in many laboratories. Comparison and analysis of sequences of unknown field isolates and variant with reference strains for establishing potential relatedness are significant advantages of sequencing (De Wit, 2000; OIE, 2008; Zulperi *et al*., 2009). RT-PCR have been applied either directly to RNA extracted from clinical samples or to eluted RNA from FTA cards. Flinders Technology Associates cards (FTA) are cotton-based cellulose membrane containing lyophilized chemicals that lyses many types of bacteria and viruses, several recent publications report the use of FTA® cards as an convenient, adequate, reliable and safe method for storing, transporting and extracting nucleic acids from inactivated infectious organisms with no interference in molecular reactions (Ndunguru *et al*., 2005; Moscoso *et al*., 2005; Purvis *et al*., 2006; Rogers and Burgoyne, 2000). In Saudi Arabia, there is very few case reports, there are little research work on the disease in the country. Nucleotide identity between isolates from Saudia Arabia, Iran and European isolates was 95% as reported (Cavanagh *et al*., 2005). Two isolates from Iran and one from Saudi Arabia isolated in 2000 had been characterized as being of the 793/B serotype by the virus neutralization test (Cavanagh, 2005). The general oligonucleotides XCE-1 and XCE2+ (Capua *et al*., 1999; Cavanagh *et al*., 1999) corresponding to nucleotides 709-1166 in the *S1* gene of the 793/B serotype were used to amplify part of S1 of the Iranian and Saudi Arabia isolates. Thus, the Iranian and Saudi Arabia isolates were clearly of the 793/B type by sequence as well as by serotype (Cavanagh *et al*., 2005). IBV 793/B type detected in swabs collected in 1997 and 1998 from Saudi Arabia, Japan, Sweden, Denmark, Poland, Italy, France and Argentina (Roussan *et al*., 2008; Asasi *et al*., 2003). The aims of this research are to performing the first constructed molecular research study on the presence of IB in Saudi Arabia through a survey study for direct detection of IBV RNA in tracheal swabs collected from commercial chicken farms in Riyadh governorate using frozen PBS and/or FTA Cards Methods. To perform further strain identification and phylogeny, the nucleotide sequences of the amplified *N* gene PCR products were determined and comparative analysis was performed to estimate the phylogenic relationship of the virus.

## MATERIALS AND METHODS

**Study area:** Samples were collected from 82 poultry house representing forty three poultry farms (16.6% of total broilers and layers poultry farms) in Riyadh Province. Samples details from different localities are summarized in Table 1.

**Samples collection and preparation:** Samples (tracheal and cloacal swabs) were collected between October 2009 and May 2010. Five birds from each poultry house (total number of samples were 930) were randomly

Table 1: Distribution of the collected samples according to localities and farm type

| | Farms distribution | | | Investigated farms | | |
|---|---|---|---|---|---|---|
| Locality | B | L | Total | B | L | No. of samples |
| Al-Kharj | 35 | 26 | 61 | 4 | 4 | 220 |
| Al-Majmua | 39 | 3 | 42 | 7 | 1 | 155 |
| Shaqra | 21 | 1 | 22 | 5 | - | 60 |
| Thadeg | 29 | 2 | 31 | 3 | 1 | 75 |
| Wadi Al-Dwaser | 8 | 1 | 9 | 1 | 1 | - |
| Al-Zolfi | - | 2 | 2 | - | 2 | 35 |
| Al-Aflaj | 9 | - | 9 | 2 | 1 | 45 |
| Al-Dwadmi | 4 | 3 | 7 | - | 1 | 30 |
| Al-Riyadh | 3 | 3 | 6 | - | 1 | 60 |
| Thorma | 4 | 9 | 13 | - | 2 | 65 |
| Al-Mozahmia | 14 | 3 | 17 | 3 | - | 80 |
| Al-Goaeaia | 7 | 3 | 10 | 2 | - | 65 |
| Hremla | 9 | 3 | 12 | 1 | 1 | 40 |
| Afif | 9 | - | 9 | - | - | - |
| Al-Oiaena | 7 | - | 7 | - | - | - |
| Al-Ghat | 2 | - | 2 | - | - | - |
| Total | 200 | 59 | 259 | 28 | 15 | 930 |

B: Broiler; L: Layer

Table 2: IBV *N* gene nucleotide sequences used in this study

| Accession No. | Virus ID | Country | Serotype | References |
|---|---|---|---|---|
| AY790350 | IBV/K210-02 | S. Korea | Arkansas | Park *et al.* (2005) |
| DQ287910 | IBV/CK/CH/LDL/98I | China | | Unpublished |
| DQ352148 | iBV/CK/CH/LDL/01I | China | | Unpublished |
| EF602445 | IBV/CK/CH/LDL/97I | China | | Unpublished |
| HQ916668 | IBV/Chicken/KSA/2010/101 | KSA | | Unpublished |
| GQ229232 | IBV/3382/06 | Taiwan | | Chen *et al.* (2010) |
| FJ888351 | IBV/H120 | Netherlands | H120 | Zhang *et al.* (2010) |
| AY514485 | IBV/California 99 | USA | California99 | Mondal and Cardona (2004) |
| FN430414 | IBV/ITA/90254/2005 | USA | | Unpublished |
| EU418975 | IBV/Arkansas/DPI | USA | | Ammayappan *et al.* (2009) |
| GQ504724 | IBV/Massachusetts | USA | | Unpublished |
| GU391029 | IBV/Jin-13 | China | | Unpublished |
| AY363968 | IBV/JP9758 | Japan | | Shieh *et al.* (2004) |
| GQ149079 | IBV/ZJ981 | China | | Unpublished |
| AY121093 | IBV/SAIBwj | China | | Unpublished |
| M28566 | IBV/Mass41 | - | | Boursnell *et al.* (1985) |
| EF602457 | IBV/CK/CH/LSD/03I | China | | Unpublished |
| EF185916 | IBV/Ind/TN/92/03 | India | | Unpublished |
| HQ916669 | IBV/Chickens/KSA/102/2010 | KSA | | Unpublished |
| DQ473615 | IBV/HB/CH | China | | Unpublished |
| DQ659366 | IBV/Brasil/TII/1975 | Brasil | | Unpublished |
| M28565 | IBV/Beaudette | - | | Boursnell *et al.* (1985) |
| AY319651 | IBV/BJ | China | | Unpublished |
| EU362619 | IBV/SD0611 | China | | Unpublished |
| EU362625 | IBV/SD0708 | China | | Unpublished |
| HQ014604 | IBV/Ma5 | China | | Unpublished |
| HM230752 | IBV/CK/CH/SD09/002 | China | | Unpublished |

selected and tracheal swabs were taken using sterile cotton-tipped swabs then soaked in 1 mL Phosphate Buffer Saline (PBS) (SIGMA®) pH 7.2 with antibiotic mixture prepared according to Burleson *et al.* (1997).

Another swabs from the same birds were imprinted over the FTA cards (Whatman® company) stored at room temperature till being used. As an alternative method for inactivation, transportation and preservation to be analyzed by Reverse-Transcriptase PCR (RT-PCR) and sequenced for the molecular characterization.

**RNA extraction and cDNA synthesis:** RNA extracted using Qiagen RNA Easy Protect Mini kit (QIAGEN®, Germany) according to manufacturing instructions. cDNA was synthesis in 20 μL reaction using transcription first strand cDNA Synthesis kit (Roche®, USA) according to the manufactures procedures.

**Polymerase chain reaction:** The primers were selected to ensure a wide detection range of IBVs according to Handberg *et al.* (1999) the universal oligonucleotide primer pairs: IBVN (+) [GAAGAAAACCAGTCCCAGAT GCTTGG] and IBVN (-) [GTTGGAATAGTGCGCTTGCA ATACCG] manufactured by TIB-MOL Biol syntheslabor Gmb H Berlin, Germany.

Amplification was done in 50 μL reaction using the following PCR profile 95°C for 3 min for initial denaturation then 35 cycles of 95°C for 30 sec (denaturation) 60°C for 1 min (Annealing) and 68°C for 3 min (Extension). Then, final extension at 68°C for 7 min. The PCR products were analyzed on 1.0% agarose gel.

**Sequence analysis:** The amplified PCR complimentary to the *N* gene products were send for direct sequencing in King Faisal Specialist Hospital and Research Center. The sequences alignment and compared to those registered in GenBank (Table 2) using MEGALIGN Program in DNAStar Laser gene 8 Software package.

## RESULTS AND DISCUSSION

**Molecular detection of IBV using RT-PCR:** Out of 82 tested poultry house in 43 poultry farms, 29 (35.36%) were shown to be positive for the detection of IBV nucleic acid using the ordinary method of sample preparation. On the other hand, only 18 (21.95%) house are shown to be positive using FTA Card Method (Table 3). Some clinical signs similar to those previously reported associated with IB infection were observed in 15.9% of poultry houses.

**Phylogenetic analysis:** Identification of homologies between nucleotide and amino acid sequences of the obtained Saudi IBV and other IBVs strains published on Gen-Bank was done using BLAST 2.0 and PSI-BLAST Search Programs (National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov), Clustal W multiple alignments of sequenced viruses and reference IBV *N* gene nucleotide sequences were carried

Table 3: Detection of IBV RNA from the whole Riyadh Province using by RT-PCR using Conventional (C) and FTA Card Preparation Method

| | | C | | FTA cards | | Total positive farm houses | |
|---|---|---|---|---|---|---|---|
| Regions | No. of farm houses tested | +ve | % | +ve | % | No. | % |
| Al-Kharj | 15 | 8 | 53.33 | 6 | 40.00 | 8 | 53.33 |
| Al-Majmaua | 15 | 4 | 26.66 | 5 | 33.33 | 7 | 46.66 |
| Shaqra | 9 | 3 | 33.33 | 4 | 44.44 | 5 | 55.55 |
| Thadeg | 7 | 3 | 42.85 | 0 | 0.00 | 3 | 42.85 |
| Northern region | 10 | 2 | 20.00 | 1 | 10.00 | 2 | 20.00 |
| Southern region (Makkah Rd) | 18 | 7 | 38.88 | 2 | 11.11 | 8 | 44.44 |
| Southern region | 8 | 2 | 25.00 | 0 | 0.00 | 2 | 25.00 |
| Total | 82 | 29 | 35.36 | 18 | 21.95 | 35 | 42.68 |

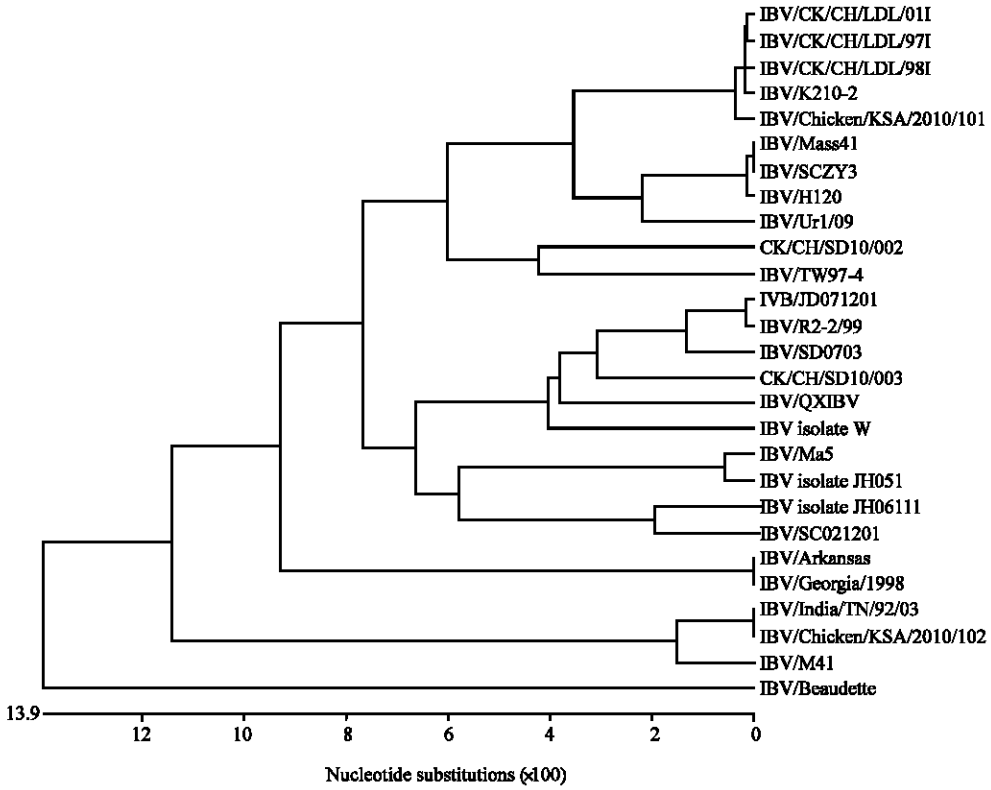


Fig. 1: Phylogenic analysis of IBV strains IBV/Chickens/KSA/101/2010 and IBV/Chickens/KSA/102/2010

out. The results showed that multiple nucleotide substitutions were observed along the length of the studied *N* gene. The sequence identity matrix developed for the sequences and reference IBV *N* gene nucleotide sequences showed identity percentages 98.9% between IBV/Chickens/KSA/101/2010, IBV/CH/CK/LDL/011 and IBV/CH/CK/LDL/981. The identity percentage between IBV/Chickens/KSA/102/2010 and IBV/India/TN/92/03 was 99.7% while the identity percentage between the sequences was 89.5%. On other hand, the percentage of identity between the sequences (IBV/Chickens/KSA/101/2010 and IBV/Chickens/KSA/102/2010) and IBV/H120 were 94.1 and 91.6%, respectively. Phylogenetic analysis clustered the IBV/Chickens/KSA/101/2010 with four IBV isolated from China while IBV/Chickens/KSA/102/2010 clustered with IBV strain isolated from India and another one isolated from China (Fig. 1 and Table 2).

Infectious bronchitis is an acute, highly contagious viral infection of chickens of all ages worldwide with adverse effects on growth and egg quality and egg production in the laying birds. It was surprising that a huge country such as Saudi Arabia with a large poultry industry has little or in fact no structured published scientific research on IB disease. Results of this study showed that 42.68% of the chickens tested were positive for IBV. This is quite high and indicates the wide spread of IBV in the Riyadh Province. However, vaccine strains may interfere with the obtained findings; virus detection accompanied with clinical signs was observed in 15.9% of tested farms. This may be due to the outdoor survival of

the virus (Ahmed *et al*., 2007). This result is comparable to that obtained from Pakistan, 57.3% in commercial poultry farms (Ahmed *et al*., 2007). In United Kingdom, 85% among commercial broiler flocks in the East and North of England were positive (Cavanagh *et al*., 1999). The 72% of tested flock were IBV positive in Iran in an epidemiological study carried out by Shoushtari *et al*. (2008). However, low detection rates were also reported such as in China (16.6%) (Zhu *et al*., 2007).

The prevalence of IBV among different localities varied from 20-55.55%. The highest percentage was reported in Shaqra and the lowest was in Northern region (Al-Zolfi, Al-Dwadmi and Hremla). This may be due the persistence of IBV infection, intermittent shedding of IBV and risk of flock to flock transmission via contamination of personnel or equipment (Cook *et al*., 1987). The high prevalence of IBV even in vaccinated poultry flocks may be due to inappropriate vaccination, vaccination strain or rapid emergence of new strains (Smati *et al*., 2002; Sharawi and Zidan, 2011) as well as environmental factors such as heat, mycotoxins and others that can induce significant stress on birds have the potential to make chickens immune-suppressed thereby increasing the chances of viral infections (Neldon-Ortiz and Qureshi, 1991; Qureshi and Hagler, 1992).

The FTA card ability to inactivate several avian pathogens has been studied previously (Perozo *et al*., 2006; Purvis *et al*., 2006). The results showed a lower detection of IBV in FTA card (21.95%) might be due to the presence of non-specific inhibitors of the Taq DNA polymerase in FTA card which could not be completely removed by RNA Extraction Method (Sharawi and Zidan, 2011) or some detrimental effects of FTA inactivation on the viral RNA as previously reported for other chemical inactivated samples (Masuda *et al*., 1999). Lower sensitivity of the RT-PCR identification of IBV in stored FTA cards was reported to be possible after 43 days was explained as a consequence of RNA denaturation (Rogers and Burgoyne, 2000; Moscoso *et al*., 2005).

Phylogenetic analysis from the 2 pools of samples collected from diseased birds showed that both IBVs sequences detected by this study were genetically diverse. The obtained nucleotide sequences IBV/CHICKENS/KSA/101/2010 shared high nucleotide sequence identity (98.9%) for Nucleocapsid (N) protein genes with IBV/CH/LDL/01I sequence a strain from China which may be revertant attenuated vaccine strains that have arisen as a result of the widespread use of IBV vaccines in the local poultry population in China (Zhang *et al*., 2005). The another nucleotide sequence IBV/CHICKENS/KSA/102/2010 sharing a high degree of identity (99.7 and 99.5%) with the IBV/INDIA/TN/92/03 and IBV/HB/CH, respectively. Results of the phylogenetic analysis for the IBVs circulating were highly diverse from the same sequences of IBV/H120 strain that commonly used as commercial vaccine for prevention of IBV in poultry flocks in KSA.

The detected IBV strains in this study were identical to most IBV strains isolated in different parts of China. It is possible that several species of birds mostly waders are known to migrate between parts of Africa and their breeding grounds in Siberia through communal stopover grounds in the African Rift Valley, the Middle East and central Asia where they are likely to come into contact with birds from the far East and that may be explained the source of the identified IBV in current research. Anyhow in the same aforementioned results; the possibility of widespread dissemination of IBV by wild birds such as wild migratory ducks. In addition, IBV has been isolated from domestic peafowl (*Pavo cristatus*), partridge (*Alectoris* sp.), guinea fowl (*Numida meleagris*), teal (*Anas* sp.) (Liu *et al*., 2005), racing pigeons (Barr *et al*., 1988) Turkeys, pigeons and parrots (Sharawi and Zidan, 2011).

## CONCLUSION

The present study proves the prevalence of IBV variants in Saudi Arabia poultry. The phylogenic analysis of the 2 IBV in this study make the ability of the existing used vaccines to contain the field strains questionable and a raise the demand for a new vaccines a national control preventive strategy to minimize the economic losses results from IBV infection in different types of chickens.

## REFERENCES

Ahmed, Z., K. Naeem and A. Hameed, 2007. Detection and seroprevalence of infectious bronchitis virus strains in commercial poultry in Pakistan. Poult. Sci., 86: 1329-1335.

Asasi, K., S.M. Seyfiabad and A. Nouri, 2003. Field study of infectious bronchitis virus in broiler using type-specific RT-PCR. Arch. Razi Instit., 55: 1-10.

Barr, D.A., R.L. Reece, D. O'Rourke, C. Button and J.T. Faragher, 1988. Isolation of infectious bronchitis virus from a flock of racing pigeons. Aust. Vet. J., 65: 228-228.

Boursnell, M.E., M.M. Binns, I.J. Foulds and T.D. Brown, 1985. Sequences of the nucleocapsid genes from two strains of avian infectious bronchitis virus. J. Gen. Virol., 66: 573-580.

Burleson, F.G., T.M. Chambers and D.L. Wiedbrank, 1997. Virology: A Laboratory Manual. Academic Press, Inc., USA.

Capua, I., Z. Minta, E. Karpinska, K. Mawditt, P. Britton, D. Cavanagh and R.E. Gough, 1999. Co-circulation of four types of infectious bronchitis virus (793/B, 624/I, B1648 and Massachusetts). Avian Pathol., 28: 587-592.

Cavanagh, D., 1981. Structural polypeptides of coronavirus IBV. J. Gen. Virol., 53: 93-103.

Cavanagh, D., 2005. Coronaviruses in poultry and other birds. Avian Pathol., 34: 439-448.

Cavanagh, D., J.P. Picault, R. Gough, M. Hess, K. Mawditt and P. Britton, 2005. Variation in the spike protein of the 793/B type of infectious bronchitis virus in the field and during alternate passage in chickens and embryonated eggs. Avian Pathol., 34: 20-25.

Cavanagh, D., K. Mawditt, P. Britton and C.J. Naylor, 1999. Longitudinal field studies of infectious bronchitis virus and avian pneumovirus in broilers using type-specific polymerase chain reactions. Avian Pathol., 28: 593-605.

Chen, H.W., Y.P. Huang and C.H. Wang, 2010. Identification of intertypic recombinant infectious bronchitis viruses from slaughtered chickens1. Poult. Sci., 89: 439-446.

Cook, J.K., A.J. Brown and C.D. Bracewell, 1987. Comparison of the hemagglutination inhibition test and the serum neutralization test in tracheal organ cultures for typing infectious bronchitis virus strains. Avian Pathol., 16: 505-511.

De Wit, J.J., 2000. Technical review. Detection of infectious bronchitis virus. Avian Pathol., 29: 71-93.

Handberg, K.J., O.L. Nielsen, M.W. Pedersen and P.H. Jorgensen, 1999. Detection and strain differentiation of infectious bronchitis virus in tracheal tissues from experimentally infected chickens by reverse transcription polymerase chain reaction. Avian Pathol., 28: 327-335.

Ignjatovic, J., S.I. Sapats and F. Ashton, 1997. A long-term study of Australian infectious bronchitis viruses indicates a major antigenic change in recently isolated strains. Avian Pathol., 26: 535-552.

Krapez, U., B. Slavec, D. Barlic-Maganja and O.Z. Rojs, 2009. Molecular epidemiology of Slovene infectious bronchitis virus strains isolated between 1990 and 2005. Proceedings of 6th International Symposium on Avian Corona and Pneumoviruses and Complicating Pathogens, June 14-17, 2009, Rauischholzhausen, Germany, pp:13-26.

Liu, S., J. Chen, X. Kong, Y. Shao and Z. Han *et al*., 2005. Isolation of avian infectious bronchitis coronavirus from domestic peafowl (*Pavo cristatus*) and teal (*Anas*). J. Gen. Virol., 86: 719-725.

Liu, S.W., Q.X. Zhang, J.D. Chen, Z.X. Han and X. Liu *et al*., 2006. Genetic diversity of avian infectious bronchitis coronavirus strains isolated in China between 1995 and 2004. Arch. Virol., 151: 1133-1148.

Masuda, N., T. Ohnishi, S. Kawamoto, M. Monden and K. Okubo, 1999. Analysis of chemical modification of RNA from formalin fixed samples and optimization of molecular biology applications for such samples. Nucl. Acids Res., 27: 4436-4443.

Mondal, S.P. and C.J. Cardona, 2004. Comparison of four regions in the replicase gene of heterologous infectious bronchitis virus strains. Virology, 324: 238-248.

Moscoso, H., E.O. Raybon, S.G. Thayer and C.L. Hofacre, 2005. Molecular detection and serotyping of infectious bronchitis virus from FTA filter paper. Avian Dis., 49: 24-29.

Murphy, F.A., E.P.J. Gibbs, M.C. Horzinek and M.J. Studdert, 1999. Classification and Nomenclature of Viruses. In: Veterinary Virology, Murphy, F.A. (Ed.). 3rd Edn., Academic Press, New York, USA., pp: 4-13.

Ndunguru, J., N.J. Taylor, J. Yadav, H. Aly and J.P. Legg *et al*., 2005. Application of FTA technology for sampling, recovery and molecular characterization of viral pathogens and virus-derived transgenes from plant tissues. Virol. J., Vol. 2. 10.1186/1743-422X-2-45.

Neldon-Ortiz, D.L. and M.A. Qureshi, 1991. Direct and microsomal activated aflatoxin B1 exposure and its effects on Turkey peritoneal macrophage functions *in vitro*. Toxicol. Applied Pharmacol., 109: 432-442.

OIE., 2008. Infectious bronchitis disease. In: OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals. pp: 443-455.

Park, J.Y., S.I. Pak, H.W. Sung, J.H. Kim, C.S. Song, C.W. Lee and H.M. Kwon, 2005. Variations in the nucleocapsid protein gene of infectious bronchitis viruses isolated in Korea. Virus Genes, 31: 153-162.

Perozo, F., P. Villegas, C. Estevez, I. Alvarado and L. Purvis, 2006. Use of FTA® filter paper for the molecular detection of newcastle disease virus. Avian Pathol., 35: 93-98.

Purvis, L.B., P. Villegas and F. Perozo, 2006. Evaluation of FTA® paper and phenol for storage, extraction and molecular characterization of infectious bursal virus. J. Virol. Methods, 138: 66-69.

Qureshi, M.A. and W.M. Hagler, 1992. Effect of fumonisin-$B_1$ exposure on chicken macrophage functions *in vitro*. Poult. Sci., 71: 104-112.

Rogers, C.D. and L.A. Burgoyne, 2000. Reverse transcription of an RNA genome from databasing paper (FTA®). Biotechnol. Applied Biochem., 31: 219-224.

Roussan, D.A., W.S. Totanji and G.Y. Khawaldeh, 2008. Molecular subtype of infectious bronchitis virus in broiler flocks in Jordan. Poult. Sci., 87: 661-664.

Schalk, A.F. and M.C. Hawn, 1931. An apparently new respiratory disease of baby chicks. J. Am. Vet. Med. Assoc., 78: 413-422.

Sharawi, S.S.A. and K.H. Zidan, 2011. Detection of infectious bronchitis virus in chickens and other avian species using reverse transcription-polymerase chain reaction. Alexandria J. Vet. Sci., 33: 87-96.

Shieh, H.K., J.H. Shien, H.Y. Chou, Y. Shimizu, J.N. Chen and P.C. Chang, 2004. Complete nucleotide sequences of S1 and N genes of infectious bronchitis virus isolated in Japan and Taiwan. J. Vet. Med. Sci., 66: 555-558.

Shoushtari, A.H., R. Toroghi, R. Momayes and S.A. Pourbakhsh, 2008. 793/B type, the predominant circulating type of avian infectious bronchitis viruses 1999-2004 in Iran: A retrospective study. Arch. Razi Inst., 63: 1-5.

Smati, R., A. Silim, C. Guertin, M. Henrichon, M. Marandi, M. Arella and A. Merzouki, 2002. Molecular characterization of three new avian Infectious Bronchitis Virus (IBV) strains isolated in Quebec. Virus Genes, 25: 85-93.

Sutou, S., S. Sato, T. Okabe, M. Nakai and N. Sasaki, 1988. Cloning and sequencing of genes encoding structural proteins of avian infectious bronchitis virus. Virology, 165: 589-595.

Zhang, Q., Z. Han, Y. Shao, J. Chen, J. Rong, S. Liu and X. Kong, 2005. Genetic variation of nucleocapsid genes of infectious bronchitis viruses isolated in China from 2000 to 2004. Virologica Sinica, 21: 575-580.

Zhang, Y., H.N. Wang, T. Wang, W.Q. Fan and A.Y. Zhang *et al*., 2010. Complete genome sequence and recombination analysis of infectious bronchitis virus attenuated vaccine strain H120. Virus Gen., 41: 377-388.

Zhu, J.G., H.D. Qian, Y.L. Zhang, X.G. Hua and Z.L. Wu, 2007. Analysis of similarity of the *S1* gene in Infectious Bronchitis Virus (IBV) isolates in Shanghai, China. Archivos Medicina Veterinaria, 39: 223-228.

Zulperi, Z.M., A.R. Omar and S.S. Arshad, 2009. Sequence and phylogenetic analysis of *S1*, *S2*, *M* and *N* genes of infectious bronchitis virus isolates from Malaysia. Virus Gen., 38: 383-391.