Indian J. Comp. Microbiol. Immunol. Infect. Dis.
Vol. 24, No. 2 (July- Dec.), 2003: 149-152

CHARACTERISATION OF AN INDIAN ISOLATE OF TURKEY POX VIRUS

Alka Singh, B.B. Dash, J. M. Kataria, S. Dandapat and K. Dhama

Division of Avian Diseases,
Indian Veterinary Research Institute, Izatnagar - 243 122 (U.P.)

(Received for publication on 24.05.2004)

A turkey poxvirus isolated from a natural outbreak of pox in turkeys was characterised using different physico-chemical agents and transmission electron microscopy (TEM) technique. The complete inactivation of the virus was achieved at 60 °C for 30 min and with the chemicals like trypsin (0.25%), phenol (0.5%) and chloroform (5%). The turkey pox virus isolate was found sensitive when exposed to the temperature of 50 °C for 30 min and to the chemicals like diethyl ether (20%) and formalin (0.5%) as the infectivity of the virus was reduced up to a considerable extent after its exposure to these agents. The electron microscopic features of the turkey pox virus was studied by examining the CAM of infected chicken embryos as well as scabs collected from turkeys infected with turkey pox virus after negative staining and the virus showed typical pox virus morphology as having characteristic oval shaped structure with dumble shaped dense core, surrounded by a double layer membrane and was found free in cytoplasm of the cell.

Avian pox diseases are contagious and slow spreading viral infections affecting numerous species of birds including chickens and turkeys (Tripathy and Cunningham, 1984). The outbreak of pox diseases in flocks of various species of the birds are quite common in many countries (Biggs, 1982; Garg *et al.,* 1984; Singh *et al.,* 2000) causing major economic losses in terms of mortality, drop in egg production and meat condemnation.

Among various bird species, turkeys are also frequently affected with poxvirus infection in India. The pox disease in turkeys have attracted attention as this disease has been reported in India as well as worldwide, resulted into severe economic losses in spite of proper management and health care (Metz *et al.*,1985; Beaudette, 1941; Bickford *et al*., 1971; Dash *et al.*, 2003). This disease causes severe diptheritic and cutaneous lesions leading to high mortality in case of diptheritic form and appreciable economic losses in case of cutaneous form of disease due to meat condemnation. (Tripathy *et al.* 1997). Pox infection in turkeys tends to be more chronic in nature with longer duration than fowl pox virus (Wakenell, 2001).

The very first time turkey pox virus (TPV) was reported by Brunett in 1934 in a turkey flock in NewYork ,Veterinary college. Several outbreaks of turkey pox virus have also been reported in turkey flocks of India (Pandey and Mallick, 1975, Dash *et al.,* 2003).

Pox disease in turkeys is caused by a Ds DNA virus of the family Poxviridae, Subfamily Chordopoxvirinae and Genus Avipoxvirus (Fenner, 1989). Avipoxvirus genus includes fowl pox (FP), turkeypox (TP), quailpox (QP), pigeonpox (PP) and canarypox (CP) as major viral strains. Fowl poxvirus represents the type species of the genus avipoxvirus (Mathews, 1982).

With the perusal of the literature, there is no information available in relation to the characterisation of turkey poxvirus so the present information in this regard will bring about the new avenues for effective management of this economically important disease of turkeys.

MATERIAL AND METHODS

Virus: Turkey Poxvirus used in the present study was isolated from a natural outbreak of pox in turkeys. The virus was adapted in the eleven days old susceptible chicken embryos through chorioallantoic membrane route up to 2nd passage. The embryo adapted turkey poxvirus was further adapted in CEF cell cultures up to 2nd passage level which was used for the physicochemical characterization studies.

Cell culture: Eleven days old chicken embryos were used for preparation of chicken embryo fibroblast (CEF) cell cultures. The cultures prepared as per the method described by Merchant *et al.* (1960) with slight modifications. Medium -199 (Microlab, Mumbai) was used for growing cells. Growth medium consisted of 10% colostrum deprived newborn calf serum whereas maintenance medium contained 2% serum.

Physico-chemical characterization : Heat treatment of turkey pox virus was done by subjecting the turkey pox virus isolate to different temperatures (50ºC and 60ºC) for subsequent periods of time (30 min) as per the method of Hamparin *et al*. (1963). Regarding chemical characterization of the turkey pox virus, the virus was treated with ether (20%) as per the method described by Andrews and Horstmann (1949) and with chloroform (5%) following the method described by Feldmann and Wang (1961). Turkey pox virus was treated with trypsin (0.25%) as per the method of Matheka *et al.* (1962) and with phenol (0.5%) as well as formalin (0.5%) as per the method of Jana (1992). After such treatments using different physical and chemical agents, the infectivity titre of the virus sample was assayed by using chicken embryo fibroblasts cell culture after proper adsorption of treated virus inoculum and was compared with untreated virus taken as control. The infectivity titres were calculated in terms of $TCID_{50}$/ml as per the method of Reed and Muench (1938).

Transmission electron microscopy (TEM): CAM of the infected chicken embryo and skin sections infected with TPV showing pock lesions were cut approx. 0.5 to 1 cm size and

processed on the line of Annuar *et al.* (1983) for the study under electron microscope. The sections were examined by TEM (JEOL, Jem-1200 Ex Electron microscope, Japan).

RESULTS AND DISCUSSION:

Turkey pox is a slow spreading viral disease causing severe economic losses in terms of meat condemnation, weight loss and drop in egg production in Indian turkey flocks. This disease has an emerging status and it was found that the past literature reveals no information with regards to characterization of this virus. Till date, TPV was considered more or less similar to FPV and the available literature reveals the physicochemical characteristics of only FPV and QPV in detail. But now it has been established using cross protection studies that TPV is different from other avipox viruses and this warrants for the characterization of turkey pox virus isolated from natural outbreak of pox in turkeys. Therefore, in this study a turkey poxvirus was isolated from a natural outbreak of pox in turkeys and was characterised using different physico-chemical agents and transmission electron microscope (TEM) technique.

Heat treatment of turkey pox virus was done by subjecting the turkey pox virus isolate to different temperatures (50 ^{o}C and 60 ^{o}C) for subsequent periods of time (30 min). The untreated virus showed the titre of $10^{7.25}$ $TCID_{50}$/ml but when the virus was exposed to 50 ^{o}C for 30 min the titre was reduced to $10^{2.5}$ $TCID_{50}$/ml, indicating that the virus is sensitive to this temperature. However, the TPV was completely inactivated at 60 ^{o}C when exposed for 30 min. The thermostability nature of the turkey pox virus was found to be similar to other avipox viruses like fowl pox virus (FPV) and quail pox virus (QPV) as reported earlier. Mc Culloch (1945) reported that fowl pox virus resisted longer than 1 hr at 50 ^{o}C. Andrews *et al.* (1978) reported that inactivation of avian pox virus occurs by heating at 50 ^{o}C for 30 min or 60 ^{o}C for 8 min. Pradhan *et al.* (1997) found that Quail pox virus could withstand 50 ^{o}C for 15, 30 and 45 min but the QPV was completely inactivated at 60 ^{o}C in 8 min.

For the chemical characterization of the turkey pox virus, the virus was treated with ether (20%), chloroform (5%), trypsin (0.25%) and some common disinfectants like phenol (0.5%) and formalin (0.5%). It was found that the infectivity of the enveloped turkey pox virus is sensitive to the effects of lipolytic agent such as chloroform but not with ether. The chloroform treatment (5%) of the turkey pox virus renders the virus to be completely inactivated but with the ether (20%) treatment turkey poxvirus was found stable showing only a decrease in the infectivity titre from $10^{7.25}$ (untreated) to $10^{4.75}$ $TCID_{50}$/ml. This shows that the virus is somewhat resistant to ether treatment but is chloroform sensitive. Similiarly, Alfalluji *et al.* (1979) reported that a pox virus isolate from a peacock was ether resistant but chloroform sensitive. Also, Pradhan *et al.* (1997) found quail pox virus to be more sensitive to chloroform than to ether treatment. Regarding other poxviruses, there are conflicting reports of sensitivity to ether and chloroform treatment. Some authors stated that some pox viruses were sensitive to both ether and chloroform but Tantawi *et al.* (1979) reported that a pigeon pox virus and its two mutants were resistant to both ether and chloroform. Randall *et al.* (1964) stated that fowl pox virus is sensitive to both ether and chloroform. Mathews (1982) reported that resistance to ether treatment is listed as one of the taxonomic criterion for pox viruses.

Trypsin in the concentration of 0.25 % at 37 ^{o}C also inactivated the TPV isolate. Chemicals such as phenol (0.5%) completely inactivated the turkey pox virus whereas formalin (0.5%) reduced its infectivity titre from $10^{7.25}$ (untreated) to $10^{3.48}$ $TCID_{50}$/ml in the present study. According to Andrews *et al.* (1978) fowl pox virus can withstand 1% phenol and 1:1000

Table 1. Effect of physico-chemical agents on turkey pox virus

Agents exposed time / Temperature	Infectivity titre of the virus (log $TCID_{50}$/ml) Untreated virus	Treated virus	Result
50 ^{o}C / 30 min.	$10^{7.25}$	$10^{2.5}$	sensitive
60 ^{o}C / 30 min.	$10^{7.25}$	0.0	inactivated
70 ^{o}C / 30 min.	$10^{7.25}$	0.0	inactivated
Ether			
20% / 8 hrs	$10^{7.25}$	$10^{4.75}$	sensitive
Chloroform			
5% / 10 min.	$10^{7.25}$	0.0	inactivated
Trypsin			
0.25% / 30 min.	$10^{7.25}$	0.0	inactivated
Phenol			
0.5% / 30 min	$10^{7.25}$	0.0	inactivated
Formaline			
0.5% / 30 min.	$10^{7.25}$	$10^{3.48}$	sensitive

formalin for 9 days. However Graham and Brandley (1940) reported that 1% suspension of the virus containing 0.25%-0.5% formalin and 0.5% phenol were completely inactivated when stored for 48 hrs at ice box temperature. Pradhan *et al.* (1997) reported that viability of quail pox virus was not affected by trypsin at a final concentration of 0.25%, But phenol had little effect on infectivity of quail pox virus whereas formalin reduced virus titre by $log10^{3.38}$. The effect of different physicochemical agents on turkey poxvirus has been illucidated in the Table-1.

The electron microscopic features of the turkey pox virus was studied by examining the CAM of chicken embryos infected with turkey pox virus as well as scabs collected from turkeys infected with turkey pox virus. The ultra structure and maturation of turkey pox virus was demonstrated by excising the pock lesions from turkey pox virus infected CAM and turkey pox virus infected scabs and studied under TEM after negative staining. The virus showed typical pox virus morphology embedded in CAM sections (Fig. 1.1) and skin samples (Fig. 1.2). The morphology of the TPV was quite clear showing characteristic oval shaped structure with dumble shaped dense core, surrounded by a double layer membrane. The viral particles could be clearly distinguishable and were found free in cytoplasm or in small groups. Some workers like Randall *et al.* (1964) found that fowl pox virus reveals virus structure as an oval/brick shaped , having average dimensions of 258X354 nm in size. Hyde *et al.* (1965) had reported that fowl pox virus is a double layered structure which agrees with findings of the present study.

Control on viral diseases is of utmost importance rather than going for treatment after an outbreak has been occurred. The informations on physicochemical characterization of a virus can help in the prevention of the mechanical/ horizontal spread of the virus. Therefore, in the present study attempts were made to know the physicochemical characterisation of the turkey poxvirus which may help for its proper management through biosecurity measures. The information generated with examination of turkey pox virus by transmission electron microscopy will help in quick identification of the virus and diagnosis of turkey pox virus of the infected field materials.

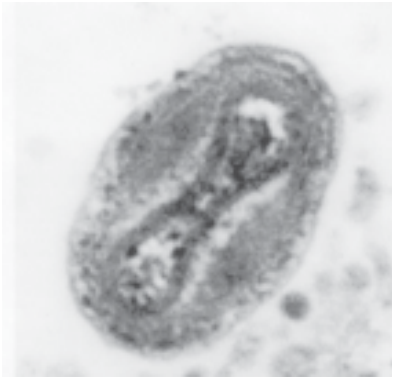

Fig. 1.1. Electron microphtograph of scab of turkey infected with turkey pox virus showing single turkey pox virus particle (61000x)

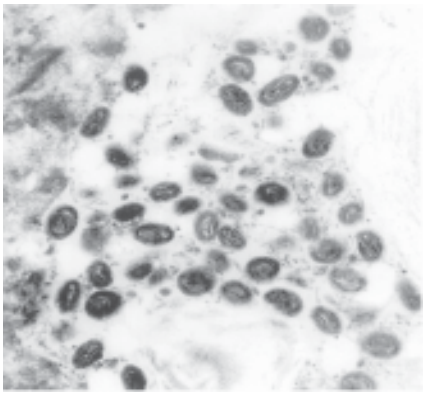

Fig. 1.2. Electron microphtograph of scab of turkey infected with turkey pox virus showing numerous turkey pox virus particle (8400x)

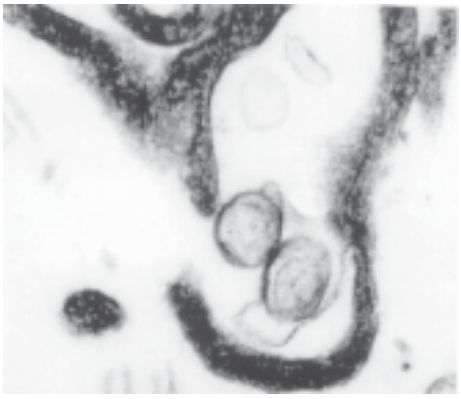

Fig. 2.1. Electron microphtograph of chorio allantonic membrane showing numerous turkey pox virus particles (15500x)

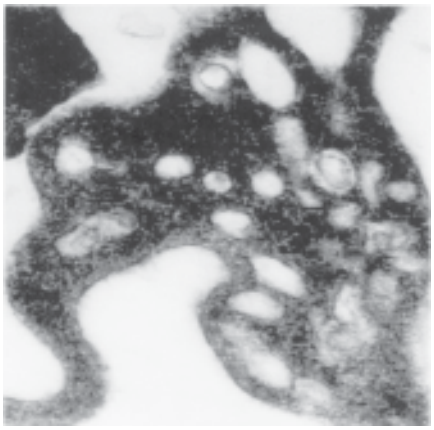

Fig. 2.2. Electron microphtograph of chorio-allantonic membrane showing turkey pox virus particles releasing from the cells (21500x)

ACKNOWLEDGEMENTS

Authors are sincerely thankful to the Director, IVRI, Izatnagar for providing necessary facilities during the research work.

REFERENCES

Alfallugi, M. M., Tantawai, H. H., Albana, A. and Alsheikhly S. (1979). *J. Wildl. Dis.*, **15**: 597.

Andrews, C.H. and Horstmann, D.M. (1949). *J. Gen. Microbiol*., **3**: 290.

Andrews, C., Pereira, H. G. and Wildy, P. (1978). Viruses of vertebrates, 4th ed. Bailliere Tindall, London, United Kingdom, **pp** 356.

Beaudette, F.R. (1941). The reasons for failures in immunisation against infectious laryngeotracheitis and pox. Proc. 45th Meet. U.S. Livestock Sanit. Assn., **p**. 127.

Bickforde, A. A., Gallina, A. M., Winterfield, R.W. and Bolte, H. (1971). *Avian dis.*, **15**: 614.

Biggs, P.M. (1982). *Avian Pathol*., **11**: 281.

Brunett, E. L (1934). *Rep. N. Y. St. Vet. Coll.*,**69:** 1932.

Dash, B. B., Kataria, J. M., Mazoomdar, S. and Dhama, K. (2003) . Avipox virus infections in Turkeys. XXI Conference of Indian Assosciation of Veterinary Microbiologists, Immunologists and Infectious Diseases (IAVMI) and National Seminar on Prospects and Challenges for Better Livestock and Poultry Health Management Employing Conventional and Molecular Approaches, Feb. 7-9, 2003 at ICAR Research Complex for NEH Region , Umiam, Barapani, Meghalaya.

Feldman, H.A. and Wang, S.S. (1961). *Proc. Soc. Exp. Biol. Med.* 106 cited by S. K. Pradhan, J. M. Kataria, K. C. Verma and S. J. Jadhao Physicochemical and biological characterization of an Indian isolate of Quail pox virus *Indian J. Comp. Microbiol. Immunol. Infect. Dis.*, Vol. **17** No.2 (July- Dec). 1996.

Fenner, F., Wittek, R. and Dumbell, K. R. (1989). The orthopox virus. *Academic press*, New york, **pp** 100.

Garg, S. K., Joshi, H. C. and Shukla, S. K. (1984). *Indian. J. Vet. Med.*, **4**: 60.

Graham, R. and Brandley, C.A. (1940). Immunisation against pox in domestic fowl. Bull. Illi. Agric. Exp. Sta. No. 470, **pp** 76 (Vet. Bull. **11**, p. 768).

Hamparin, V. V., Hilleman, M. R. and Ketler, A. (1963). *Proc. Soc. Expt. Biol. and Med.*, **112**: 1040.

Jana, D. (1992). Studies on characterisation of virus isolated from an outbreak of cowpox in India. M. V. Sc. thesis. in veterinary virology submitted to Deemed University, IVRI. Izzatnagar (UP) India.

Matheka, H. D., Mayr, A. and Bogel, K. (1962). Zentabl. Bakt. Parasitkde I. Orug. **187**: 137.

Mathews, R.E.F. (1982). *Intervirology.*, **17**: 42.

Mc Culloch, E.C. (1945). Disinfection and sterilisation. Lea and Febiger, Philadelphia.

Merchant, D.J., Kahn, R.H. and Murphy, W.H.J. (1960). *Handbook of cell and orgen culture.* Durgess. Pub. Co., Minnesota.

Metz, A. L., Hatcher, L., Newman, J. A., Halvorson, D. A. (1985). *Avian Dis*., **28**: 850.

Pandey, K. D. and Mallick, B. B. (1975). *Indian J. Animal Health*. **14** : 99 cited by S. K. Pradhan, J. M. Kataria, K. C. Verma and S. J. Jadhao Physicochemical and biological characterization of an Indian isolate of Quail pox virus *Indian J. Comp. Microbiol. Immunol. Infect. Dis.*, Vol. **17** No. 2 (July-Dec). 1996.

Pradhan, S.K. (1997) Characterisation of a Quail pox virus (Coturnix *coturnix japonica).* M.V.Sc. thesis submitted in veterinary virology submitted to Deemed university of IVRI (UP).

Randall, C.C., Gafford, L.G., Darlington, R.W. and Hyde, J. (1964). *J. Bacteriol*., **87** : 939.

Reed, L. I. and Muench, H. (1938). *Amer. J. Hyg.*, **27** : 493.

Singh, P., Kim, T. J. and Tripathy, D. N. (2000). *Avian Pathol.*, **29**: 449.

Tantawi, H. H., Al Falluji, M. M. and Shony, M. O. (1979). *Acta Virol.*, **23**: 249.

Tripathy D. N. and C. H. Cunningham. (1984). *Avian pox. In Diseases of Poultry*. 8th ed. M. S. Hofsted, H. John Barnes, B. W. Calnek, W. M. Reid, and H. W. Yoder, Jr. ed, Iowa State University Press, Ames, IA.

Tripathy, D.N. and W.M. Reed. Pox. (1997). In : *Diseases of Poultry* , 10th ed. B. W. Calnek, H. J. Barnes, C. W. Beard, L. R. Mc Dougald and Y. M. Saif, eds. Iowa State Univ. Press, Ames. **pp**. 643.

Tripathy, D. N., Singh, P. and Schnitzlein, W. M. (1998). Fowl pox : A re-emerging disease in chickens. Proc : 49th North central Avian Dis. *Conference and Symposium on Enteric and Emerging Diseases,* Indian Poles, In. **pp** 36

Wakenell, S., Patricia, D.V.M, Ph.D., Diplomate A.C.V.P. By courtesy of Vineland laboratories (2001). Avian pox virus. 'Zoo Technica' *World's Poultry Journal,* **pp**. 48.