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THE SOURCE OF AVIAN PARAMYXOVIRUSES ISOLATED DURING AN OUTBREAK OF INFLUENZA AMONG CHILDREN

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Summary. — In 1986 five avian paramyxovirus (PMV) strains were isolated in embryonated chicken eggs from sick children with influenza. The strains were identified as PMV-2 serotype due to the close antigenic relationships between their HN-proteins and of the reference PMV-2 strains isolated from different birds all over the world. No seroconversion to the isolates was found in the sick children, however, HI-antibodies were detected in hen's sera, eggs of which were used for the new strains isolation. The possible origin of isolated PMV-2 viruses is discussed.

Key words: *avian paramyxoviruses*; *antigenic properties*; *embryonated chicken eggs*

It is known that avian paramyxoviruses are widely spread (Isashenko *et al.*, 1975; Lvov *et al.*, 1977; Alexandrova *et al.*, 1972; Tůmová *et al.*, 1979; Alexandrova *et al.*, 1983). According to the antigenic structure of the haemagglutinin (HA) and neuraminidase (NA) they can be divided into 9 serotypes (Alexander *et al.*, 1983). In the nature, PMV predominantly infects feral and domestic birds. Further investigations have shown the possibility of interspecies transfer of PMV strains to mammals. The evidence for this fact is supported by the finding that guinea pigs are susceptible to avian PMV-2 (Yucaipa) (Fleury *et al.*, 1983) and by the presence of antibodies to Yucaipa virus in human sera in Senegal (Fleury *et al.*, 1984). Isolation from pigs of a strain which is antigenically closely related to avian PMV-3 serotype (Lipkind *et al.*, 1986) can also evidence for circulation of avian PMV among mammals. The possibility of transovarial transmission was suggested when a virus antigenically similar to the parainfluenza 2 virus was isolated from chick embryonated eggs (Wagner, 1966). This consideration is very important because chicken eggs are often used as a sensitive system for isolation of human and animal ortho- and paramyxoviruses. It explains our interest in 5 haemagglutinating viruses isolated from embryonated eggs in Murmansk virological laboratory; the chicken embryos were infected with nasopharyngeal swabs collected from affected children, during increase of influenza morbidity in 1986.

Antigenic properties of the new isolates were studied in following tests: haemagglutination inhibition (HI), neuraminidase inhibition (HI), double immunodiffusion (DID) according to Palmer *et al.* (1975). Antisera against avian PMV 1—9 serotypes and the new strains were prepared as described (Yakhno *et al.*, 1969; Tůmová *et al.*, 1979).

Preliminary identification has shown that neither of the new isolates was inhibited in HI-test with any influenza A, B, C antiserum. The special test with nitrous acid (Granoff *et al.*, 1961) has indicated that investigated isolates belong to paramyxoviruses. Further investigations in HI and NI tests

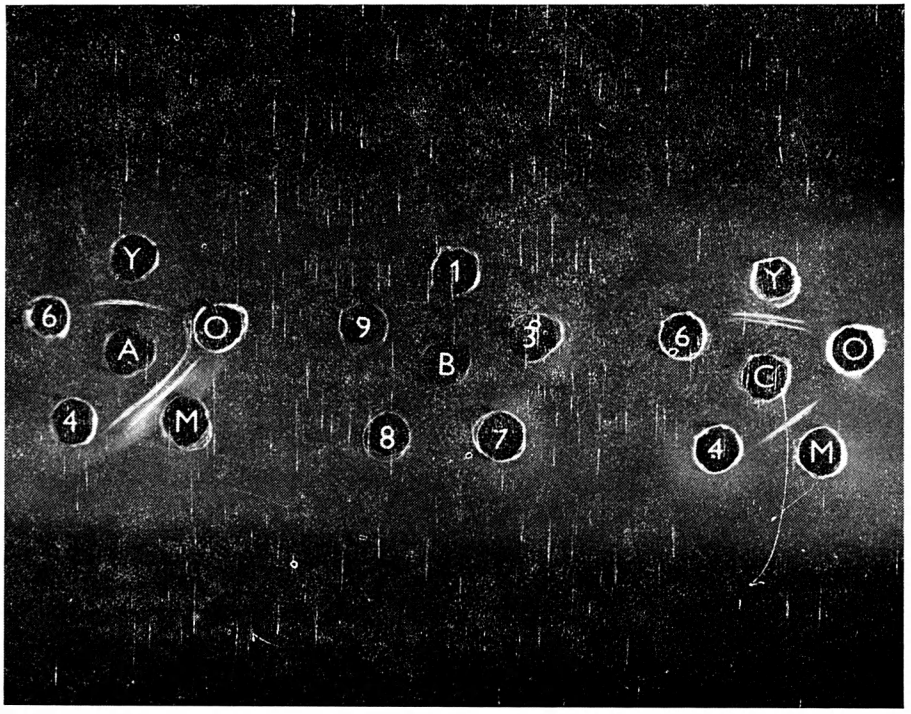

Fig. 1

Double immunodiffusion test (DID) in agarose gel
In the centre: A, B — antigens PMV-2/USSR/Murmansk/3/86
C — antigen PMV-2/Chicken/California/Yucaipa/56
Wells around: Y — antiserum — PMV-2/Chicken/California/Yucaipa/56
O — antiserum to PMV-2/Troglodytes/Olomouc/77
1—4, 6—9 — different serotypes of avian PMV
M — antiserum to PMV-2/USSR/Murmansk/3/56
Antigens PMV-2/Chicken/California/Yucaipa/56 and PMV-2/USSR/Murmansk/3/86 react clearly but less intensively as with homologic antiserum. Weak line between antigen PMV-2/USSR/Murmansk/3/56 and antiserum PMV-2/Troglodytes/Olomouc/77 indicates differences in their antigenic properties or lower precipitation activity.

with specific antisera to various human and animal parainfluenza types 1—3, avian paramyxoviruses 1—9 serotypes have demonstrated that the new isolates reacted only with the antisera to avian paramyxoviruses type 2 (PMV-2). The results obtained in DID-test also confirmed a very close relationship between the isolates and the reference strains of PMV-2 serotype. The data shown in Fig. 1 demonstrate that the new isolate No. 3 reacts only with the antiserum to PMV-2/chicken/California/Yucaipa/56.

The new strains were isolated after inoculation of embryonated chicken eggs with nasopharyngeal swabs from the children with influenza [the sera of these children have seroconversion to influenza A virus (H1N1)]. The presented results raise the question about the origin of the new viruses. There are several possibilities to explain the source of these strains. It could be the sick children, despite of the fact, that HI — antibodies to PMV-2 were not demonstrated. The absence of antibody in children's sera cannot exclude foregoing PMV infection as the first contact with some infectious agents lead usualy to transient antibody increase below the limit of detectability by customary methods. (Profera and Palladino, 1986.)

Another source of infection may be chicken embryos themselves. The virus can get into embryos by different ways: 1) by direct contamination with laboratory PMV strains; 2) by infections of the egg's shell at the passage through the cloaca; 3) by transovarial virus transfer from the sick hens. The first possibility can be excluded because there are no PMV strains on stock in virological laboratory of Murmansk. More likely source of the virus are the chicken embryos which can become infected when passing through the cloaca or as a result of transovarial transfer. This possibility is supported by PMV-2 antibody demonstrated in sera of egg-laying hens and is well correlated with the data shown by French *et al.* (1967) and Hanson (1978). The isolations of PMV from embryonated chicken have been demonstrated many years ago by Andrewes and Glover, (1984).

We report of this observation to call virologist's attention to chicken embryos used for virus isolation as these may incidentally become the source of viruses unusual in man.

References

Alexander, D. J., Shortridge, K. F., Collins, M. S., and Chettle, N. J. (1979): Properties of a newly isolated serologically distinct avian paramyxovirus. *Arch. Virol.* **6**, 105—118.

Alexander, D. J., Hinshow, V. S., Collins, M. S., and Yamane, N. (1983): Characterization of viruses which represents further distinct serotypes (PMV-8 and PMV-9) of avian paramyxoviruses. *Arch. Virol.* **73**, 29—36.

Andrewes, Ch., and Glover, R. E. (1944): The influenza "A" outbreak of October-December 1943. *Lancet* **2**, 104—105.

Fleury, K. J. A., Vincon, Y., Demiinere, C., Cailland, F., Paix, M. A., Babin, M., Bonnici, J. F., Faivre, R., and Du Pasquier, P. (1983): Susceptibility of the guinea pig to paramyxovirus Yucaipa. *Annales de Virologie* **134 E**, 429—432.

Fleury, K. J. A., Bonnici, J. F., Babin, M., Du Pasquier, P., and Sant, A. (1984): Serological evidence of human infection with the paramyxovirus Yucaipa in Senegal, West Africa. *Am. J. trop. Med. Hyg.* **33**, 190—191.

French, E. L., Sr. George, T. D., and Percy, J. J. (1967): Infection of chicks with recently isolated Newcastle disease virus of low virulence. *Australian Vet. J.* **43**, 404—409.

Granof, A. (1961): Induction of Newcastle disease virus mutant with nitrous acid. *Virology* **13**, 402—408.

Hanson, R. P. (1978): *Newcastle Disease in Diseases of Poultry*, 7th edition the Iowa State University Press, Hofstad *et al.* (Ed.) pp. 619—656.

Isashenco, V. A., Yakhno, M. A., Osidge, H. G., and Sheboldov, A. V. (1975): Detection of myxoviruses of Yucaipa group among agents cause chick respiratory disease. In *Ecology*, Moscow, pp. 67—72 (in Russian).

Lipkind, M., Shoham, D., and Shihmanter, E. (1986): Isolation of a paramyxovirus from pigs in Israel and its antigenic relationships with avian paramyxoviruses. *J. gen. Virol.* **67**, 427—439.

Lvov, D. K., Syurin, V. N., Nikiforov, L. P. *et al.* (1977): Discovery of Natural Foci of Newcastle Disease Virus in the U.S.S.R. *Vopr. Virusol.* **22** (3), 311—316 (in Russian).

Palmer, D. F., Coleman, D. T., Dowdle, W. R., and Schild, G. C. (1975): Advanced laboratory Technique for influenza diagnosis. *Immunology Series No. 6*, US Depart. Hlth. Educ. Welth. CDC, Atlanta, Ga, pp. 107—129.

Profeta, M. L., and Palladino, G. (1985): Serological evidence of human infections with avian influenza viruses. *Arch. Virol.* **90**, 355—360.

Tůmová, B., Štumpa, A., Janout, V., Uvízl, M., and Chmela, J. (1979): A further member of the Yucaipa group isolated from the common wren (*Troglodytes troglodytes*). *Acta virol.* **23**, 504—507.

Wagner, K., and Enders-Ruckle, G. (1966): Ein in Hühnerei vorkommendes Parainfluenza-Virus. *Zbl. Vet.* **13 B**, 127—130.

Yakhno, M. A., and Zakstelskaya, L. Ya. (1969): Production of antisera for Parainfluenza Type 4 A and 4 B viruses. *Vopr. Virusol.* **14** (6), 745—746 (in Russian).