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PATHOGENIC ASSESSMENT OF CHICKEN EMBRYO FIBROBLAST CELL CULTURE ADAPTED INFECTIOUS BRONCHITIS VIRUS VACCINE*

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Infectious bronchitis (IB) is a major cause of economic losses in the poultry industry worldwide. Passage of the presently available egg adapted vaccines (seed materials) against five subtypes of IBV mass serotype viz., M5, M41, M48, H52 and H120 in chicken embryo fibroblast cell culture (CEFCC) was attempted. The effect of serial passages of IBV mass serotype chicken embryo adapted (CE adapted) vaccines in CEFCC, with regards to pathogenicity was assessed both qualitatively and quantitatively in embryonated chicken eggs (ECE) and TC system. The results were compared with those of the "start up" viruses.

Materials and Methods

The test subtypes were purchased from private pharmaceuticals (M_5 - Nobilis, M_{41} - Fort Dodge Animal Health, M_{48} - Hester Pharmaceutical Limited, H_{52} - and H_{120} - Lohman). The TC media were procured from HiMedia Lab (India). *Clostridium perfringens* type A culture was obtained from microbial type collection centre 450 (MTCC), Chandigarh, India.

Freeze dried live vaccine viruses were propagated ten times in nine-day-old ECE from serum antibody negative (SAN) chicken flock by allantoic route. The allantoic fluid (A/F) served as "start up" virus for adaptation of the corresponding virus in CC system, after confirming the potency, both qualitatively and quantitatively.

CEFCC monolayers were prepared and the "start up" virus of the five IBV mass serotypes was passaged in the cell cultures. The presence of virus was assessed by observation of specific lesions in embryonated chicken eggs (ECEs). The passages, which evinced cytopathogenic effect (CPE) were also confirmed for the virus presence in ECE. Ten such passages were undertaken, serially in CEFCC, employing the previously passaged virus as "start up" virus. All the "active passages" of the corresponding subtypes were microtitrated by "simultaneous infection" technique.

The revised seed viruses and the alternative TC passaged viruses were titrated in ECE by preparing $\log_{10}$ dilutions using allantoic fluid (A/F) of each subtype. EID_{50} was calculated using Reed and Muench formula (Reed and Muench, 1938). HA titres of the TC passaged subtypes of IBV mass serotype at every passage level was assessed by using both, A/F and TC fluid of corresponding passages. Micro HA

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(Gough *et al.*, 1992) and rapid plate HA tests were conducted after duly treating the viruses with *Clostridium perfringens filtrate* as per the corresponding standard procedures (Ruano *et al.*, 2000).

Results and Discussion

Specific lesions of IBV, viz., dwarfing, curling and diffuse haemorrhages of embryo and oedematous amnion, either individually or combined were observed in all the "start up" viruses of the cell culture, thus confirming the potency of the "start up" viruses. In addition, abdominal enlargement and prolapse of the internal organs were observed significantly in case of Holland subtypes (H_{52} and H_{120}). Specific lesions observed in ECE tallied with findings of Verma and Malik (1971).

All the five subtypes of IBV Mass serotype vaccines responded to rapid plate haemagglutination (HA) within one minute after due treatment with *Clostridium perfringens* filtrate enzyme for 120 minutes at 37°C, using 5 per cent chicken RBC. The TC fluids of all the subtypes of IBV mass serotype also responded to rapid plate HA. The positive response of the "start up" virus subtypes (Ruano *et al., loc. cit*) and TC passaged viruses to rapid plate HA test also confirmed the presence of the virus.

The titres (EID_{50}) of the "start up" viruses in ECE ranged from 6.4 $\log_{10}$ (M_5) to 7.7 $\log_{10}$ (H_{52}) per 0.1 ml (Table 1). The mean titres (EID_{50}) of TC passaged viruses were lowered ranging from 2.9 $\log_{10}$ (M_5) to 3.7 $\log_{10}$ (H_{52}) per 0.1 ml, in case of "blind" passages during initial stages of adaptation, the titres (EID_{50}) increased gradually, reaching maximum titre ranging from 5.5 $\log_{10}$ (M_5 and H_{120}) to 6.0 $\log_{10}$ (M_{48}) per 0.1 ml at the tenth passage. During alternative passages of the virus in ECE and CC (CK) system, Cowen and Hitcher (1975) encountered similar decreased titres ($10^{4.6}$ to $10^{5.8}$ per 0.1 ml) in case of "blind" passages and subsequent increase in the titre of $10^{6.4}$, when the virus could initiate CPE in the CC system.

The virus titre in TC system (TC infective dose 50% $TCID_{50}$) of tenth passage of all the subtypes ranged from 4.2 $\log_{10}$ (H_{120}) to 5.1 $\log_{10}$ (M_{48}) per 0.1 ml (Table 1), as against the lower titres of the active passages, ranging from 2.9 $\log_{10}$ (H_{120}) to 3.9 $\log_{10}$ (H_{52}) per 0.1 ml. Meilin *et al.*, (1998) observed stable TCID50 titres of the CEF propagated IBV-NH, T, H_{52} and M_{41} subtypes, the titres ranging from 10^6 to $10^{7.7}$ ml^{-1}. They also confirmed that CPE in CEF was caused by IBV, using electron microscopy and HI tests.

Duration of occurrence of CPE reduced with increasing passages. Based on the optimal culture time and the amount of virus propagation, the viable virus particles released into the medium reached maximum, between 36-72 h (Meilin *et al., loc. cit*). Cowen and Hitcher (*loc. cit*) reported 75 per cent CPE at 48 h post infection. HA titres of the startup viruses were 9 $\log_2$ (512) - M_{48}, 8 $\log_2$ (256) - H_{120}, 7 $\log_2$ (128) - M_{41} and 6 $\log_2$ (64) - M_5 and H_{52}. HA titres of the TC passaged subtypes showed considerable variations in HA titres (Table 2) (King, 1984).

Summary

Adaptation of prevailing embryonated chicken egg (ECE) adapted infectious bronchitis mass serotype vaccine viruses in chicken embryo fibroblast cell culture (CEFCC) was attempted. Presence of virus was assessed qualitatively (observation of

Table 1 : Quantitative assessment of pathogenicity - virus titers (log_{10}) in embryonating chicken eggs and chick embryo fibroblast cell culture.

Level of passage in TC system / "start up" viruses	*In vivo* - virus titer in embryonating chicken eggs - (EID_{50})					*In vivo* - microtitration in chick embryo fibroblast cell culture system - ($TCID_{50}$)				
	M_5	M_{41}	M_{48}	H_{52}	H_{120}	M_5	M_{41}	M_{48}	H_{52}	H_{120}
Startup virus	6.4	6.5	7.6	7.7	6.8	–	–	–	–	–
I P	–	–	–	–	–	–	–	–	–	–
II P	2.9	3.4	3.1	3.7	3.0	–	–	–	–	–
III P	–	–	–	–	–	–	–	–	–	–
IV P	3.7	4.9	3.5	4.1	3.3	–	–	–	–	–
V P	–	–	–	–	–	3.2	–	–	3.9	–
VI P	4.7	4.7	4.6	4.8	3.8	3.5	3.5	3.7	3.9	–
VII P	–	–	–	–	–	3.9	4.0	4.2	4.2	2.9
VIII P	5.3	5.4	5.3	5.3	5.0	4.2	4.3	4.5	4.4	3.7
IX P	–	–	–	–	–	4.2	4.9	4.8	4.5	4.0
X P	5.5	5.8	6.0	5.9	5.5	4.4	5.0	5.1	4.7	4.2

Table 2 : Haemagglutination titers (log_2) of IBV "start up" and CEFCC passaged viruses.

Level of passage in TC system / "start up" viruses	Haemagglutination titers (log_2) of IBV "start up" and CEFCC passaged viruses.				
	M_5	M_{41}	M_{48}	H_{52}	H_{120}
"Startup" virus	6	7	9	6	8
I P	2	3	4	2	2
II P	2	3	4	2	2
III P	2	3	4	2	2
IV P	3	3	4	2	2
V P	3	4	5	3	2
VI P	3	5	6	2	3
VII P	2	5	5	2	4
VIII P	2	4	5	2	3
IX P	–	3	4	–	2
X P	–	3	4	–	2

specific lesions in ECEs and specific cytopathic effect in active passage levels) and effect on pathogenicity was assessed quantitatively (titration in ECE and microtitration in CEFCC system). In addition, presence of virus was confirmed by micro haemagglutination (HA) and rapid plate HA tests. However, the high protective titres of the corresponding "startup" viruses could not be achieved. Hence, few more passages in TC system may be advocated, thus enabling to achieve an effective pentavalent vaccine, against IB.

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