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Note

Comparison of pathogenicity and immunogenicity of two strains (TCND and Montana) of Newcastle disease virus propagated in four tissue cell types. (*)

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SUMMARY

Four different kind of cell (pig kidney line, Bovine kidney line, lamb kidney sub-culture and pig kidney sub-culture) were used to determine the degree of their susceptibility to TCND (Bankowski vaccine strain) and the Montana N.D. Strains to compare their pathogenicity and immunogenic properties in chickens.

The results of cytopathic effect (C.P.E.) hemadsorption and acridine orange staining (A.O.) with both strains of virus showed 4 plus C.P.E. and 4 plus hemadsorption in the PK line and PKs.c.

The pathogenicity of Montana strain was more severe to the Bovine kidney cell line than the pig kidney cell adapted TCND strain. The lamb K.sub. culture showed to be less susceptible to invasion by both strains.

The titers of TCND vaccine decreased in the following, order PK. line, PKs.c., L.K.S.C. and Bovine kidney line cells. Vaccines produced in the 4 cell cultures were diluted to have an equivalent final titers ($10^{5.7}$ ELD50).

The birds vaccinated with TCND vaccines did not show any clinical signs of the disease during the period of post vaccinal observation.

All of the vaccinated chickens which were challenged, 4 weeks later with 0.2 ml. of the virulent N.D. virus containing 200,000 ELD50 resisted against the challenging dose.

Serological test (H.I. Titer) before and after vaccination showed no spreading of the Bankowski strain to unvaccinated chickens which were kept in the same unit with the TCND.

INTRODUCTION:

Newcastle disease (ND) appeared in Indonesia in 1926 and in spring of the

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same year was reported by Doyle in Newcastle, England. The presence of the disease was recognized in the State of California by Beach in 1943 and by Beaudette and Black in other states in 1945. For almost nine years the infection was called differently, chicken flue, nine day pneumonia and or pneumoencephalitis in California. The conditions under which the virus was introduced to other countries from Java are not known. There is no information as to primary host of NDV. (10-11) Since recognition of NDV in Java in 1926 most of the poultry scientists in the world have worked on propagation, nature of the virus, morphology, composition, antigenicity, Immunogenicity, thermostability, pathogenicity and methods of the control by using different vaccine types. These methods were as follows:

1. Control by inactivated virus vaccines

Beach (1944) investigated the disease in California and showed that inactivated virus vaccines gave good protection against paralysis, mortality and some resistance against a drop in egg production. but the vaccine did not protect the infection of the respiratory system.

2. Live virus vaccines

Beaudette criticized the use of inactivated vaccines because inactivated vaccines did not give complete protection against the disease and he believed that the live virus vaccines gave superior protection (1949. The live vaccine immunized all susceptible birds, regardless of age, following a single administration which produced an immunity for a long time.

The B1 strain was recognized by Hitchner and Johnson in 1946.

Since B1 strain was relatively safer than Beaudette's live virus strain it was accepted by most vaccine manufacturers and became available to the industry in 1950.

The B1 strain could be used safely for vaccination of baby chickens, and stimulated a good immunogenic response in older birds, it was used for birds of all ages. The B1 strain has very low potential for spreading from chick to chick.

3. Use of other lentogenic strains for vaccination

In 1952 another lentogenic strain of virus, i.e. the La Sota strain was introduced as a commercial vaccine. These vaccines were recommended to be used by the intramuscular route.

The La Sota and F strains gave better protection than the B1 strain when immunity was challenged with virulent Newcastle virus (Winterfield 1957).

4. Tissue culture vaccine (TCND) or Bankowski, strain (11)

Tissue culture vaccine propagated in pig kidney cell was commercially available in 1960. The strain of virus attenuated from the virulent Calif 11914 strain by serial passage in a tissue culture system and named TCND by Bankowski in 1958. The major advantage of the tissue culture vaccine is that it does not spread from vaccinated to

unvaccinated chickens and that it is grown on non avian tissues which prevents the transfer of other transovarian avian viruses. e. g. Leukosis, Encephalomyelitis, Mycoplasmosis, Salmonellosis, CELO virus and perhaps others.

In order to find a Newcastle vaccine free of the above diseases the virus must be propagated in sufficiently high titer in tissues other than of avian origin.

The purpose of this study was to determine the degree of susceptibility of cultures of 4 cell lines to TCND and the virulent Montana strain of ND virus and to compare their pathogenicity and immunogenic properties in chickens.

MATERIALS AND METHODS

Virus: Three strains of Newcastle disease (ND) virus were used. Strain Montana was originally isolated in 1947, by Dr. Bankowski, from tissue of chickens, in the state of Montana, having the disease.

The TCND virus used was V-108 commercially obtained from Poultry Health Laboratories in Hela Cells which was originally modified by the author (RAB) (1,2,3,5)

The GB Texas strain NDV was used for challenge. The virus was propagated in embryonating chicken eggs, and the allantoic and amnionic (aafl) were pooled, distributed in 2- ml amounts and stored at 9°C. The challenge dose given by intramuscular route in all cases, was 200,000 ELD50.

Cell cultures: Four different cell cultures, pig kidney line (PKL), pig kidney sub-culture (PK s.c.), lamb kidney sub-culture (LK s.c.) and bovine kidney line (BKL) were prepared in leighton tubes. The growth medium used for pig kidney sub-culture (PK s.c.) lamb kidney sub-culture (LK s.c.) was Earls medium with yeast and lactalbumin (EYL) with 10% ox serum. Eagles minimum essential medium with 5% bovine serum was used for the pig kidney line (PKL) and bovine kidney line cells (BKL) (1,2).

Chickens: The white Leghorn chickens in these experiments were obtained from the Department of Poultry Husbandry, University of California (6,8). The flock has not been vaccinated against any disease during the past 15 years. These chickens divided in 3 groups:

1— Four groups of 10 chickens each, were inoculated (40) with 0.25 ml of TCND prepared in each of the four cell cultures. A group of 10 chickens was held as unvaccinated controls. All birds were kept in 1 isolation unit.

2— Four groups of 3 chickens each, were inoculated with 0.25 ml of the Montana strain propagated in each of the 4 cell cultures.

A group of 8 chickens was used as uninoculated control and the birds were held in a separate and well isolated unit.

3— Group of 10 chickens was used as uninoculated control in an isolated unit.

All of these birds were challenged at the end of the experiment with 0.2 ml of GB strain of ND Virus which contained 200,000 ELD 50.

RESULTS

Results of the cytopathic effect. (CPE) hemadsorption, and staining with acridine orange (A.O) of the two strains of ND Virus (TCND and Montana) in the 4 cell cultures after 24,48,72 and 96 hours is summarized in Table I.

Table I

Results of C.P.E. hemadsorption and stain A.O. after 24, 48, 72 and 96 hours

Virus strain	Cell cultures	Observations							
		24 hours		48 hours		72 hours		96 hours	
		C.P.E.	A.O. and hemad.	C.P.E.	A.O. and hemad.	C.P.E.	A.O. and hemad.	C.P.E.	A.O. and hemad.
TCND	PKSC	+	+	+++	+++	++++	++++	++++	++++
	PK Line	-	-	+	+	++++	++++	++++	++++
	BK Line	-	-	++	+	++	+	+++	++
	LKSC	-	-	+	+	++	++	+++	+++
Montana	PKSC	-	-	++	+	+++	+++	++++	++++
	PK Line	-	-	++	+	++++	+++	++++	++++
	BK Line	-	-	+	+	+++	++	++++	++++
	LKSC	-	-	+	+	+++	+++	+++	+++

1. 24 hours after inoculation

Some CPE (one plus) and some hemadsorption was observed (using 0.4% chicken red blood cell in buffered saline) with the TCND strain in PK sub-cultured cells.

There was neither a cytopathic effect, nor hemadsorption with the Montana strain nor with TCND in any other cell cultures at this time.

2. 48 hours after inoculation

Syncytium, hemadsorption, packing of vesicular nuclei in all four cell cultures were observed with TCND and the Montana strain, however, the PK subcultured cells appeared to be more susceptible particularly with the tissue culture adapted TCND strain.

3. 72 hours after inoculation

There were CPE, hemadsorption, karyorrhexis of nuclei in the syncytia, hyperplasia in the PK s.c., LK s.c. and PK line cultures with the TCND strain of virus. The reactions were more severe in the PK cells than in the bovine or lamb cell cultures.

The Montana strain also showed CPE hemadsorption, packing of vesicular nuclei, red ball stage of degeneration in all of the cell cultures .

4. 96 hours after inoculation

The CPE and hemadsorption with both strains of virus (4 plus CPE 4 plus hemadsorption) was more severe in the PK line and PK s.c. The Montana strain was also more pathogenic in the BK line cells than the PK cell adapted TCND strain. The LK s.c. appeared to be less susceptible to invasion by both strains.

TITRATION OF THE SUPERNATANT FLUIDS IN CHICKEN EMBRYOS TO DETERMINE ELD50

A pool was made of the 72 and 96 hour supernatant fluids of the cultures and titrated in 9 day old chicken embryonated eggs. After centrifugation, 0.1/ml of the TCND and Montana vaccines were inoculated into the allantoic sac (1, 4). The time of the death following inoculation with the TCND strain varied between 72 to 120 hours and between 48 to 72 hours with the Montana strain. Hemorrhages were found in the dead embryos caused by TCND but were more severe in the dead embryos caused by strain Montana.

As shown in Table 2 titer of $10^{7.7}$ to $10^{8.24}$ were obtained with strain Montana in the 4 cell cultures. TCND produced lower titers in PK cells ($10^{6.24}$ per 0.1 ml). The lowest infectivity titers were obtained with the TCND strain propagated in the bovine and lamb kidney cells ($10^{5.7}$ per 0.1 ml).

The hemagglutination (HA) activity of the allantoic and amnionic fluids (aafl) in the dead embryos caused by TCND were uniformly positive but strain Montana often killed embryos without showing HA of the aafl.

Table 2
Titration in chicken embryos of TCND and Montana strains of NDV propagated in 4 cell cultures

Virus strain	Cell culture	Titer ELD50 per 0.1 ml
TCND	PK sc.	$10^{6.24}$
	PK line	$10^{6.24}$
	Bovine K line	$10^{5.7}$
	Lamb K sc	$10^{5.7}$
MONTANA	PK sc.	$10^{7.7}$
	PK line	$10^{8.7}$
	Bovine K line	$10^{8.24}$
	Lamb K sc.	$10^{8.24}$

VACCINATION OF CHICKENS WITH VACCINES PREPARED IN THE 4 CELL CULTURES (2,6)

Each of the titrated tissue culture propagated virus suspensions were appropriately diluted to $10^{5.7}$ ELD50 to contain equal concentration of the virus. The virus suspensions were immediately used as vaccines in the following experiment:

80 susceptible birds divided into 5 groups:

1— A group of 40 chickens was vaccinated with the TCND vaccines. (10 chickens inoculated with PK s.c., PK line, BK line and LK s.c. respectively).

2— A group of 10 unvaccinated chickens was held in the same unit with the TCND vaccinated as contact controls.

3— A group of 12 chickens was used for Montana vaccines. (3 chickens were vaccinated with each of the virus suspensions grown in the 4 cell cultures).

4— A group of 8 unvaccinated chickens was placed in contact with the Montana strain vaccinated chickens.

5— A group of 10 unvaccinated chickens was held in a separate isolation unit

and used as challenge controls at the end of the experiment.

Vaccination: Each of the chickens vaccinated with the respective virus suspensions containing $10^{5.7}$ ELD50 received 0.25 ml intramuscularly.

RESULTS

1. HI titer before vaccination

All of the birds were bled to determine the presence of HI antibodies (7,8) (degree of passive immunity) before vaccination at 21 days of age. All of the birds were HI negative.

2. Clinical signs after vaccination

a. The chickens that were inoculated with strain Montana showed depression, rales beginning with the 72nd hour which was followed by severe paralysis and death. All of the birds died 15 days after vaccination.
b. All of the unvaccinated hen mate contact controls held with the Montana vaccinated group died 17 days following the vaccination date.
c. There were no signs or symptoms in the group of chickens inoculated with TCND vaccine during the 1 weeks post vaccination observation period.
d. No clinical signs or serological response was found as evidence of spreading of the TCND vaccine during the 4 weeks period in the contact control chickens housed with the vaccinated chickens.

IMMUNOLOGICAL RESPONSE (HI TITER) IN CHICKENS VACCINATED WITH THE TCND VACCINES GROWN IN 4 CELL CULTURES (9)

1. Pig kidney sub-culture propagated TCND

The birds vaccinated with TCND propagated in PK s.c. showed a high titer by hemagglutination inhibition test. The HI titer (GMT) was 111 (Fig. 1 and Table 3).

2. Pig kidney line propagated TCND

The serological response in chickens to the TCND virus propagated in pig kidney line cells were highest of the 4 groups. The GMT of the 10 chickens was 120. (Fig. 1).

3. Lamb kidney s.c. and Bovine kidney line propagated TCND

The results of the HI response in chickens vaccinated with TCND propagated in lamb and bovine cells were lower.

The GMT values were 78 and 70 respectively. (Fig. 1 and Talbe 3).

CHALLENGE OF VACCINATED CHICKENS (6,7,8,9)

Twenty nine days after vaccination, all chickens vaccinated with the TCND

Table 3

Immunological response of four groups of chickens vaccinated at 21 days of age with TCND virus propagated in four cell cultures and response of the chickens to intramuscular challenge at 7 weeks of age*

Group** No.	No.of birds	HI titer (GMT) Before vaccination at 21 days	HI titer (GMT) 29 days following vaccination	% survival after challenge at 7 weeks of age
1	10	0	120	100
2	10	0	111	100
3	10	0	78	100
4	10	0	70	100
5	10	0	0	0
6	10	0	0	0

* All chickens were resistant to challenge with 200,000 ELD50 of strain GB NDV.

** 1— Pig kidney line
2— Pig kidney sub-culture
3— Lamb kidney sub-culture
4— Bovine kidney line
5— Contact control
6— Control

vaccines ,the contact controls (10 chickens), and the group of unvaccinated control chickens which were held separately from the vaccinated groups were challenged. The challenge dose of GB virus for each bird consisted of 0.2 ml of a virus suspension diluted to contain 200,000 doses of ELD50. (0.2 ml of $10^{5.0}$ ELD50).

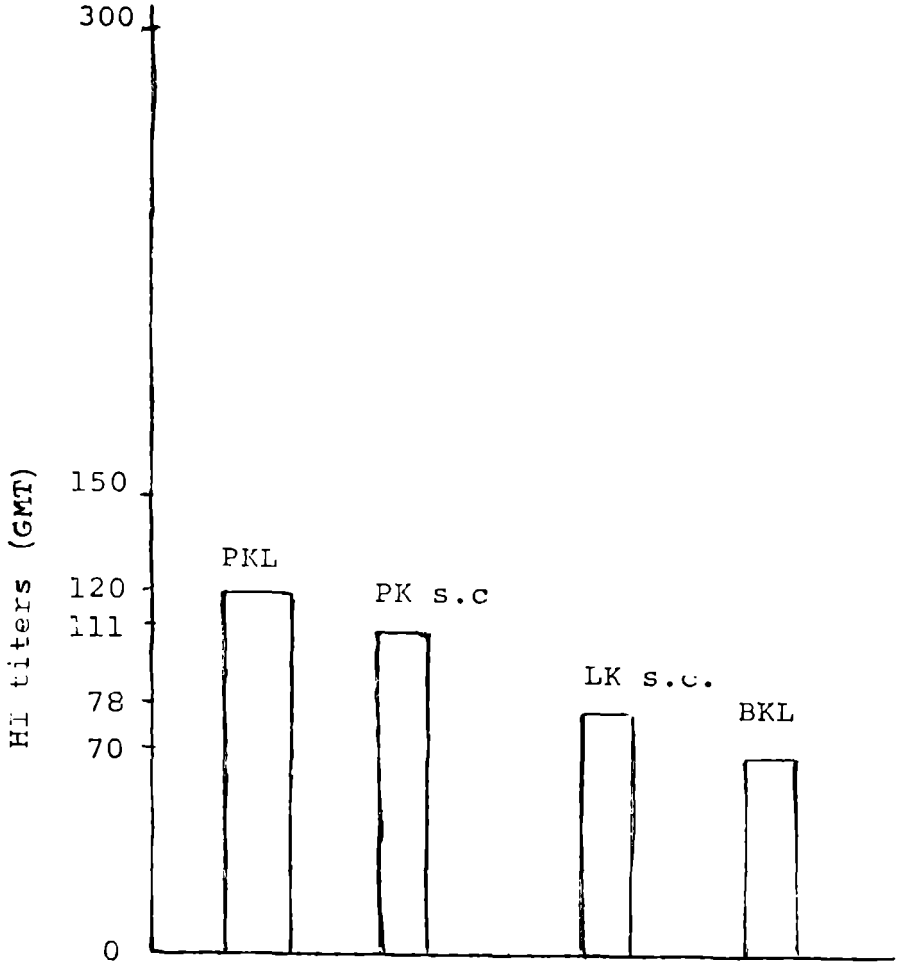
The results were as follows:

1. All chickens vaccinated with 0.25 of TCND vaccines resisted the virulent GB virus.
2. All of the TCND contact control chickens died within 6 days.
3. All of the unvaccinated control chickens died within 6 days.

CONCLUSIONS

Since live virus vaccines prepared in avian eggs are capable of transferring egg borne viruses such as Leukosis, Encephalomyelitis, Celo virus, Mycoplasmosis and Salmonellosis, Dr. Bankowski introduced a tissue culture modified vaccine propagated in non avian tissues to correct this disadvantage. The vaccine was available commercially in 1960. Studies were conducted concurrently with 2 strains of ND culture, Pig kidney line, Bovine kidney line, Lamb kidney sub-culture and Pig kidney sub-culture.

Fig. I: HI titers (GMT) in chickens 29 days after vaccination at 21 days of age with strain TCND virus propagated in 4 cell cultures.*



*All chickens were resistant to challenge with 200,000 ELD50 of strain GB virus.
PKL = Pig kidney line
PKSC = Pig kidney sub-culture
LKSC = Lamb kidney sub-culture
BKL = Bovine kidney line

The cytopathic effect (CPE), hemadsorption and reactions to acridine orange staining were similar with both viruses on all cell cultures but the time of appearance of the reactions varied (Table 1). The infectivity titers of the 2 viruses for chicken embryos varied and titers of TCND decreased in the following order: PK line, PK s.c., LK s.c. and BK line cells (Table 2)

Vaccines were prepared from each of the 2 viruses which were propagated in the 4 cell cultures. Each vaccine was diluted to be of equivalent infecting potency ($10^{5.7}$ ELD50) for chicken embryos before use in 3 weeks old susceptible chickens.

The birds which were vaccinated with 0.25 ml amounts of the vaccine prepared with the Montana strain and as well as the hen contact controls died 17 days after vaccination.

The birds vaccinated with TCND vaccines did not show any clinical signs of disease during a 4 weeks observation period following vaccination.

Twenty nine days after vaccination all birds were challenged IM with 0.2 ml of virulent strain of GB virus containing 200,000 ELD50 doses of virus.

None of the vaccinated chickens showed signs of ND following challenge with the virulent virus.

Serological test (HI test) before and after vaccination showed that there was no spreading of the TCND vaccine virus to unvaccinated controls which were kept in the same unit with the TCND vaccinated chickens. All of the contact control chickens died after challenge with the virulent GB virus indicating their susceptibility to ND during the 4 weeks period.

Vaccines prepared with strain TCND in 4 cell cultures produced a good antibody response in vaccinated chickens and protected the chickens against 0,2 ml. of GB virus containing 200,000 ELD50 doses.

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