NEWCASTLE DISEASE VIRUS: SOME BIOLOGICAL CHARACTERISTICS OF TWELVE SAMPLES ISOLATED IN BRAZIL

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ITO, N.M.K.; PRESTES, A.A.; NICIPORCIUKAS, M.C. Newcastle disease virus: Some biological characteristics of twelve samples isolated in Brazil. Rev. Fac. Med. vet. Zootec. Univ. S. Paulo, 23(1): 47-53, 1986.

SUMMARY: Twelve Brazilian isolates of NDV were studied through evaluation of their biological properties. Three lentogenic, three mesogenic and six velogenic strains of NDV were identified according to their ICPI. They were differentiated from one another by the comparison of the thermostability, elution time and ability to agglutinate horse RBC. Among the lentogenic strains, two isolates resembled the LaSota vaccinal strain and one the B1 strain. None of the isolates resembled the 2C Ulster strain. The mesogenic sample obtained from turkeys could be differentiated from the two mesogenic chicken isolates. The velogenic sample were divided into 4 groups: rapid eluters - thermostable - (+)HA for horse RBC (one sample); rapid eluters - thermostable - (-)HA for horse RBC (3 samples); rapid eluters - thermosensible - (-)HA for horse RBC (one sample); slow eluters - thermosensible (-)HA for horse RBC (one sample).

UNITERMS: Newcastle disease virus; Haemo-agglutination;* Elution; Isolation; Pathogenicity; Thermostability

INTRODUCTION

The first description in Brazil of a Newcastle disease (ND) outbreak was reported by SANTOS et alii, 11 (1954). ND still is a serious poultry farm problem in the country despite the fact that various vaccination schedules have been adopted. During the 1971-1979 period ND was one of the most prevalent diseases in Brazil (OLIVEIRA & GIRAO, 10, 1979; OLIVEIRA et alii, 9, 1981). Some of the ND virus (NDV) field isolates were classified as velogenic viscerotropic strains (BORDIN et alii, 3, 1976; GUIMARAES, 4, 1982) and velogenic neurotropic strains (BORDIN et alii, 3, 1976). Although their biological characteristics related to the hemagglutinin (HA) activity were not described.

HA related studies-thermostability, elution and HA of horse red blood cells (RBC) - are very important to differentiate one strain from another (HANSON, 6, 1975). SPALATIN et alii, 13 (1970), proposes the thermostability of the HA of NDV as a strain marker in epizootiologic studies.

Considering these data and aiming a better understanding of the different populations of NDV in the country, we decided to study some aspects of the pathogenicity and HA activity related properties in 12 Brazilian field isolates.

MATERIAL AND METHODS

Virus: The strains B1 (Hitchner) and LaSota, were originated from commercial vaccines (Salsbury). The 2C Ulster strain was supplied by Dr. J.B. Mcferran from Veterinary Research Laboratory, Stormont, Northern Ireland. The field samples of NDV were isolated from lung and trachea of vaccinated chickens and "backyard birds". The samples isolated from 1977 to 1979 were chosed by chance from a stock of 52 samples previously isolated by Dr. N.M.K. Ito. During 1983, all birds showing respiratory symptons were submited to the necropsy and virus isolation was attempted. All the positive isolations were included in this study. The field isolate called MC was provided by Dr. M.C. Galetti, Campinas, São Paulo, Brazil.

Stock viruses were propagated by chorioallantoic route in 9 to 11 day-old embryonated chickens eggs from a specific pathogen-free source (Granja Rezende, Uberlândia, MG, Brazil). The allantoic fluid harversted 72 hours post-inoculation from embryos killed at 4 C was clarified by centrifugation at 1500 rpm for 5 minutes and then stored at - 20 C in 1 ml vials until tested.

Chickens. Commercial Hybro day-old chickens were obtained from the Cooperativa

Agricola de Cotia and tested to the presence of NDV antibodies by the hemagglutination inhibition (HI) test before their use in experiments. Chickens hatched from the same source were raised in isolation until 4-6 weeks of age.

Haemagglutination (HA) test. The HA test was carried out according to the micromethod technique using double dilutions of the stock virus (1/2, 1/4, 1/8,.....) and 0,5% of chicken or horse red blood cells (RBC) suspension (HANSON, 6, 1975). The HA titers were expressed as mean of the reciprocal of virus dilution (log). The HA specificity was checked using anti-NDV hyperimmune chicken serum.

Virulence testes. The intracerebral pathological index (ICIP) test and the intravenous pathological index (IVPI) testes were carried out as described by ALLAN et alii, 2 (1978).

The embryo mean death time (EMDT) was assayed by the inoculation of 9 to 11 day-old chicken embryos, via the chorioallontoic cavity with serial ten fold dilutions (10^{-1} , 10^{-2} , ...) of the NDV isolate. The B1, LaSota and 2C Ulster strains of NDV were used as controls. The inoculated eggs were candled at 12 hour-intervals up to 5 days post-inoculation. The EMDT induced by each virus preparation was calculated for the highest dilution related with death of all embryos (NATIONAL ACADEMY OF SCIENCES, 8, 1971). The EMDT to the isolates which did not kill all the inoculated embryos (e.g. lentogenic isolates) was represented by the mean value obtained by the following formula:

(Dead Embryos x death time) + (Survivor Embryos x 72 hours)/total of inoculated embryos.

Hemagglutination (HA) stability. One ml vials each virus sample were immersed in a 58 C water bath and removed at different time intervals (5, 10, 15, 20, 30, 60 and 120 minutes). The samples were chilled immediately after removal, and titration of HA activity was performed through the standard HA test using chicken RBC (HANSON, 6, 1975)

Elution time. Time of elution NDV isolates was assayed using the HA micromethod test (HANSON, 6, 1975). After adding 0.5% of chicken RBC suspension to the serial two-fold dilution of each virus samples, the first HA result was read at the time when control cells sedimentation has occurred. Then the microtest plate was kept at 4 C for 24 hours and another reading was performed after shaking the plate to ressuspend the RBC. After approximately 2 hours the last HA reading was done. At the same time B1, LaSota and 2C Ulster strains of NDV were assayed.

The elution time was considered slow when a clear hemagglutination pattern was observed in all readings. When positive HA was seen only in the first reading the time of elution was said rapid (HANSON, 6, 1975).

RESULTS

Twelve Brazilian field NDV samples, isolated form different poultry flocks are related in Table 1. Two of the 1977 samples were isolated from vaccinated broilers showing 2% of mortality, respiratory and digestive signals. The birds of the 3120 case showed a very high HI titer (109.14 ± 1.80) typical of field disease. The other sample (3200) was obtained from vaccinated layers at the peak of production showing egg drop and mild respiratory signals. The samples isolated in 1978 were recovered from birds with respiratory signs. One was isolated from non-vaccinated adult turkeys (3362) while the other one was obtained from vaccinated 30 week-old layers having a quite high HI antibody response. The isolate 3589 was recovered in 1979 from vaccinated 6 week-old broilers showing digestive symptoms and a very high morbidity and mortality. At the same year MC - Campinas was isolated from a severe outbreak of ND by Dr. Galleti. During 1983 we had five positive isolations. Three samples were obtained from vaccinated broilers with about five to eight weeks of age presenting varied symptoms. One sample was isolated from non-vaccinated "backyard chickens" with a high HI antibody titer (31.7 ± 2.8). The last isolate was obtained from a 29 - week-old vaccinated broilers breeder with egg drop.

Table 2 shows biological aspects of three non-pathogenic isolates: ICPI lower than 0.40, IVPI equal to zero and EMDT superior to 87 hours were detected. They were all able to agglutinate chicken RBC and only two were able to agglutinate horse RBC. The heat stability of the HA was of 5 minutes and the elution time was rapid for only one sample, while the other two had slow elution time.

All the non-pathogenic samples tested did not kill 100% of the inoculated embryos.

LaSota strain had an ICPI of 0.17, agglutinated horse RBC, was thermolabile and the elution time was slow. The B1 and 2C Ulster strains had the same ICPI (= 0), they both did not agglutinate horse RBC, but differed in the heat stability of the HA (5 and 180 minutes respectively) and in the elution time (B1 was a rapid eluter and 2C Ulster was a slow eluter).

Results of nine pathogenic isolates sequenced according to the ICPI are shown in Table 3. The 3362, 5132 and 3589 isolates had a lower ICPI (0.93 to 1.44) when compared to the other six samples 3107, 5022, 5088, 5050, MC - Campinas and 3120 (1.62 to 1.79). The low ICPI isolates

showed IVPI of 1.37, 0.78 and 0.47 respectively while the high ICPI samples had an IVPI ranging from 1.20 to 1.55. Almost all the pathogenic samples killed the embryos very quickly (at least, until 48 hours) excluding the 3362 and 3589 isolates which had an EMDT around 72 hours.

Also high HA titers were detected against chicken RBC. Only the 3362 and 5050 samples were able to agglutinate horse RBC and there was a wide range of results in the heat stability test (5 to 120 minutes). The rate of elution was mainly rapid except for the samples 3362 and MC - Campinas that were slow eluters.

DISCUSSION

HANSON, 6 (1975) proposed the classification of the NDV samples as lentogenic, mesogenic or velogenic pathotypes based on the ICPI and/or IVPI and/or EMDT. The samples listed in the table 2 according to the ICPI and IVPI can be considered lentogenic. All the lentogenic field isolates as well as the vaccinal strains presented a very similar EMDT as preconized for the non-pathogenic samples (SPALATIN & HANSON, 12, 1976; WESTBURY, 14, 1979).

The properties related to the HA activity have been used to differentiate lentogenic samples of NDV (SPALATIN et alii, 13, 1970; HANSON, 6, 1975; SPALATIN & HANSON, 12, 1976), as well as to identify subtrains cloned by the plaque morphology method (LOMNICZI, 7, 1976, YACHIDA et alii, 15, 1979). The 3412 and 3200 isolates did not present any detectable difference, since they were both slow eluters, thermolabile after 5 minutes of treatment and agglutinated horse RBC. Comparing these results with the lentogenic vaccinal strains of NDV (Tab. 2) we can conclude that the 3200 and 3412 isolates might be the LaSota strain since they share similar responses in the HA related tests. Then we could think that the respiratory distress and/or egg drop found in the layers (Tab. 1) were not associated with the illness state of the flock. The 5354 lentogenic isolate also could not be associated with the field disease found in the 6 week-old broilers (Tab. 1), because it has the same HA properties of B1 strain (Tab. 2). Probably all the lentogenic isolates are vaccinal virus since LaSota and B1 strains of NDV are widely used in the country. Although a small number of lentogenic samples have been tested, it is very interesting the fact that we did not find any isolate similar to the 2C Ulster strain.

Nine isolates were considered pathogenic on basis of their ICPI (Tab. 3). According to the scheme for pathotyping NDV (HANSON, 6, 1975), ICPIs ranging from 1 to 1.75, chicken embryo deaths occurring at 48 to 72 hours and absence of intense diseases symptoms in chickens inoculated by conjunctival or cloacal route characterize mesogenic strain. ALEXANDER & ALLAN, 1 (1974) found an ICPI approximately 0.9 of the mesogenic Beaudette C strain of NDV. So the 3362, 5132 and 3589 isolates can be classified as mesogenic strains, in spite of the high IVPI (1.37) found for the first sample. ALEXANDER & ALLAN, 1 (1974) called attention to the fact that for some strains of NDV, IVPI evaluation does not always serve as a good guide to the overall disease picture and mortality of birds infected with each strain.

The 3362 isolate was obtained from non-vaccinated 77 week-old turkeys showing respiratory signals (Tab. 1). This strain was distinct from the two other broiler isolates (5132 and 3589) because it shows differences in the three HA parameters (Tab. 3). Another curious fact is related with the field observations in the case 3589. The broiler flock showed a very high mortality (10%) at the 6th. week of age just after the spray vaccination (Tab. 1). So we believe that the high mortality observed for this mesogenic sample in the flock is due to the coincidence of the field virus challenge "versus" spray vaccination. In the case 5132, 2% of mortality was detected. The vaccination schedule was different and the spray vaccination was not used.

High ICPI (1.62 to 1.79) and IVPI (1.20 to 1.55), EMDT lower than 48 hours, were the characteristics of the other six samples of NDV (3107, 5022, 5088, 5050, MC - Campinas, 3120) (Tab. 3). The velogenic strains of NDV induced an over-lapping of nervous, digestive and respiratory signs when 6-week-old chickens were inoculated intravenously. Gut hemorrhages were also seen (MC - Campinas and 5088). As a consequence we could not reconize the two pathological forms reported by HANSON, 5 (1972) (viscerotropic or neurotropic-velogenic forms).

The velogenic isolates of NDV (Tab. 3) can be separated in 4 distinct groups of virus which differ in some aspects related to the hemagglutination properties. Thus the first group is represented by the 5050 isolate which did not agglutinate equine erithrocytes. Thermostable hemagglutination and fast elution time were additional characteristics. Five isolates unable to agglutinate equine erithrocytes (3107, 5022, 5088, MC - Campinas, 3120) can be separated in three distinct groups: thermolabile hemagglutination/rapid eluter 5022; thermolabile hemagglutination/slow eluter-MC - Campinas; thermostable hemagglutination/rapid eluter - 3107, 5088 and 3120.

The analysis of Tab. 1 related with

the data presented in Tab. 3 shows some interesting facts. The velogenic 5050 isolate which was recognized above as a distinct strain was obtained from non-vaccinated "backyard chickens". This fact represents a serious threat to the poultry farming since this chickens may act as velogenic virus reservatories.

MC-Campinas was isolated from a serious outbreak of ND in 1979 and considered as a highly pathogenic strain (Galleti - personal comunication). Four years latter (1983) we could not detect any sample resembling the MC strain. On the other hand if we take the 1977 broiler velogenic isolates (3120 and 3107) six years latter we found out a similar strain (5088). Unfortunately we do not know if samples resembling the 5022 and 5088 strains were detected before 1983. Anyway with this simple study we believe to have provided some additional information about NDV epidemiology in our country where the disease still represents a serious sanitary problem.

ITO, N.M.K.; PRESTES, A.A.; NICIPORCIUKAS, M.C. Virus da doença de Newcastle: Algumas características biológicas de 12 amostras isoladas no Brasil. Rev.Fac.Med.vet.Zootec.Univ.S.Paulo, 23(1):47-53, 1986.

RESUMO: Foram estudadas algumas propriedades biológicas de 12 amostras de vírus da Doença de Newcastle (VDN), isoladas no Brasil. De acordo com o índice de patogenicidade intracerebral (IPIC), três amostras foram consideradas lentogênicas, três mesogênicas e 6 velogênicas. As amostras foram diferenciadas entre si pela comparação da termoestabilidade, tempo de eluição e a habilidade de aglutinar hemácias de cavalo. Dentre as amostras lentogênicas dois dos isolados se assemelharam à amostra vacinal LaSota e um à amostra B1. Nenhum dos isolados se assemelhou à amostra 2C Ulster. O isolado mesogênico obtido de perus pode ser diferenciado dos dois outros isolados mesogênicos obtidos de galinhas. As amostras velogênicas foram divididas em 4 grupos: eluição rápida - termoestabilidade - HA (+) para hemácias de cavalo (uma amostra); eluição rápida-termoestabilidade - HA (-) para hemácias de cavalo (três amostras); eluição rápida - termosensibilidade - HA (-) para hemácias de cavalo (uma amostra); eluição lenta - termosensibilidade - HA (-) para hemácias de cavalo (uma amostra).

UNITERMOS: Doença de Newcastle, vírus; Hemoaglutinação; Eluição; Isolamento; Patogenicidade; Termoestabilidade

TABLE 1 — Source of the newcastle disease virus field isolates related with year of isolation, age of the chickens, clinical signs and vaccination schedule

Year of isolation	Number	Chickens Type	Age (weeks)	Signs	Morbidity (%)	Mortality (%)	HI titre (GMT antilog) ± S.D.	Vaccination Virus/Schedule
1977	3107	Broiler	7	Respiratory/digestive	10	2	Not done	B1/4th day(eye) + LaSota/14th and 30th day (eye)
	3120	Broiler	5	Respiratory/digestive	50	2	109.14-1.80	B1/1st day(eye) + LaSota/14th day (eye)
	3200	Layer	32	Respiratory/egg drop	40	0	Not done	LaSota, B1 - Sechedule ommited
1978	3362	Turkey	77	Respiratory	unknown	unknown	Not done	Not done
	3412	Layer	30	Respiratory	20	0	7.40±2.48	LaSota/25th, to th and 100th day(oral)
1979	3589	Broiler	6	Digestive	50	10	Not done	B1/5st day (eye) + LaSota/18th and 35th day (spray)
	MC-Campinas	Broiler	unknown	Digestive/Respiratory/nervous	unknown	unknown	Not done	unknown
1983	5022	Broiler	5	Digestive/nervous	40	1.5	Not done	B1/8th day (oral) + LaSota/31st day (oral
	5050	"Backyard chicken"	4	Respiratory,nervous Digestive	100	100	31.7±2.8	Not done
	5088	Broiler breeder	29	Egg drop	10	0	Not done	LaSota, B1 and oil emulsion vaccins schedule ommitted
	5132	Broiler	8	Respiratory	10	2	3.71±1.90	B1/7th day (oral) + LaSota/28th day (oral
	5354	Broiler	6	Digestive	10	2.3	2.0±0	B1/5th day (oral) + LaSota/25th (oral)

TABLE 2 - Biological properties of non pathogenic Newcastle disease virus isolated from poultry flocks in Brazil

Laboratory	Controls			NDV Isolates		
	LaSota	B_1	2C	3200(a)	3412	5354
Chicken pathogenicity						
ICPI	0.17	0	0	0.16	0.27	0.40
IVPI	ND	ND	0	0	0	0
Egg pathogenicity						
LD_{50}	$10^{5.5}$	$10^{8.7}$	$10^{6.5}$	$10^{8.7}$	$10^{6.5}$	$10^{7.0}$
EMDT	≥ 87.0	≥105.6	≥91.20	≥98.4	≥105.6	> 110.4
HA Test						
Chicken RBC	256	256	64	128	32	64
Horse RBC	128	0	0	128	128	0
Heat stability(b)	5	5	180	5	5	5
Elution	Slow	Rapid	Slow	Slow	Slow	Rapid

(a) Number of case
(b) Minutes at 56oC
EMDT - Embryo mean death time
ICPI - Intracerebral pathogenic
ND - not done
IVPI - Intravenous pathogenic index
LD_{50} - Lethal dosis 50 per cent
RBC - Chicken red blood cells

TABLE 3 - Pathogenic samples of NDV isolated from poultry flocks in Brazil sequenced according to their ICPI

Laboratory Tests	ISOLATES								
	3362	5132	3589	3107	5022	5088	5050	MC/Cam-pinas(a)	3120
Chicken pathogenicity									
ICPI	0.93	1.30	1.44	1.62	1.65	1.68	1.72	1.77	1.79
IVPI	1.37	0.78	0.47	1.47	1.40	1.28	1.20	1.55	1.33
Egg Pathogenicity									
LD_{50}	$10^{8.0}$	$10^{8.5}$	$10^{7.0}$	$10^{10.5}$	$10^{9.2}$	$10^{10.0}$	$10^{10.0}$	$10^{12.3}$	$10^{10.0}$
EMDT (hours)	75	48	73.20	43.2	36.0	36.0	36.0	48.0	48.0
HA Test									
Equine-RBC	16	0	0	0	0	0	64	0	0
Chicken-RBC	64	64	16	128	128	64	64	128	128
HEAT STABILITY (minutes at $46^{o}C$)	5	45	60	90	5	30	60	15	120
Rate of elution	Slow	Rapid	Rapid	Rapid	Rapid	Rapid	Rapid	Slow	Rapid

(a) - Field NDV isolate supplied by Dra. Maria do Carmo Galetti - Campinas - SP
LD50 - Lethal dosis 50 per cent
EMDT - Embryo Mean Death Time
HA test - Hemagglutination test
RBC - Red blood cells
ICPI - Intracerebral pathogenicity index
ICPI - intravenous pathogenicity index

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Recebido para publicação em: 12/08/85
Aprovado para publicação em: 09/04/86
Impresso em 11/86