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Antigenic and Functional Properties of Hemagglutinin-Neuraminidase Glycoprotein of Avian Paramyxoviruses

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TABLE OF CONTENTS

A. EXECUTIVE SUMMARY

B. INTRODUCTION

C. RESEARCH OBJECTIVES

D RESEARCH ACCOMPLISHMENTS

1 Viruses Used in the Studies

2 Basic Properties of the APMV Hemagglutinin-Neuraminidase (HN) Glycoprotein

2 1 Thermostability of the HN glycoprotein of the APMV prototype strains

2 2 Antigenic characterization of the Enders-Ruckle virus (ERV)

2 3 Physico-chemical and functional properties of the HN of the APMV-2 ("Yucaipa-like") viruses
2 3 1 Elution from the red blood cells
2 3 2 Electrophoretic mobility of the APMV-2 structural properties

2 4 Antigenic heterogeneity by the HN antigen amongst the APMV-2 viruses
2 4 1 Antigenic heterogeneity amongst the APMV-2 reference strains studied by hemagglutination inhibition (HI) cross-reaction test
2 4 2 Antigenic heterogeneity amongst the APMV-2 strains isolated in Israel
2 4 2 1 Isolation and identification of the APMV-2 viruses isolated in Israel in 1979-81
2 4 2 2 Antigenic heterogeneity amongst the APMV-2 viruses isolated in Israel in 1979-81 HI cross reaction tests
2 4 2 3 New isolations of the APMV-2 viruses in Israel from domestic and wild birds after decade of silence
2 4 3 *Discussion and conclusions Ecological and evolutionary enigmas endemic source or "import"?*

2 5 The first isolation of the APMV-4 in Israel antigenic peculiarities of the local isolates

2 6 Isolation of two different APMV serotypes in the cases of mixed infection of domestic and wild birds in Israel
2 6 1 Mixed infection by APMV-1 and APMV-2 viruses
2 6 2 Mixed infection by APMV-1 and APMV-4 viruses

2 7 Virological surveillance and serological identification of the hemagglutinating agents isolated in Kenya
2 7 1 Field surveillance of avian population in 1994 poultry and feral birds
2 7 2 Serological identification of hemagglutinating agents isolated in Kenya in 1994 from domestic birds
2 7 3 Field surveillance performed during the visit of the PI to Kenya in 1995 and isolation of hemagglutinating agents

3 Analysis of the APMV-1 and APMV-2 Viruses by Means of Monoclonal Antibodies (MAB) against the Viral Envelope Proteins

3 1 Preparation of the MABs against the viral envelope proteins of NDV and Yucaipa viruses
3 1 1 Preparation of purified virus material and its fractionation into the envelope and internal viral proteins

3 1 2 Immunization of BALB mice with the viral envelope proteins
3 1 3 Production of hybridoma cell lines
3 1 4 Determination of the antibody activities of the clones obtained by the ELISA
3 1 5 Determination of the MAB isotypes

3 2 Characterization of the MABs against the NDV Establishment of the MAB panels
3 2 1 The MAB panel against the matrix (M) protein determination of the non-overlapping groups
3 2 2 The MAB panel against the fusion (F) protein determination of the non-overlapping groups
3 2 3 The MAB panel against the hemagglutinin-neuraminidase (HN) protein determination of the non-overlapping groups
3 2 4 The MAB-mediated analysis of the NDV strains isolated in Israel from domestic and wild birds analysis of contrasting variability amongst the strains

4. Sequencing of APMV Genes

4 1 The strategy

4 2 The methodical approach

4 3 Sequencing of particular APMVs' genes
4 3 1 Enders-Ruckle virus
4 3 2 APMV serotype 1 (APMV-1) APMV-1/racing pigeon/Israel/84
4 3 2 1 HN gene
4 3 2 2 L gene
4 3 3 APMV serotype 3a (APMV-3a) the prototype strain APMV-3a/turkey/Wisconsin/65
4 3 3 1 P gene
4 3 3 2 F gene
4 3 3 3 L gene – the END of the genome
4 3 4 APMV serotype 3b (APMV-3b) the prototype strain APMV-3b/parakeet/Netherlands/449/75
4 3 5 PMV-3/swine/Nazareth/80 antigenically similar to the strain APMV-3b/parakeet/Netherlands/75
4 3 6 APMV serotype 6 (APMV-6) the prototype strain APMV-6/duck/Hong Kong/199/76
4 3 7 APMV serotype 7 (APMV-7) the prototype strain APMV-7/dove/Tennessee/75
4 3 7 1 M gene
4 3 7 2 F gene
4 3 7 3 HN gene
4 3 8 APMV serotype 8 (APMV-8) the prototype strain APMV-8/goose/Delaware/76
4 3 9 APMV serotype 9 (APMV-9) the prototype strain APMV-9/duck/New York/80 HN gene
4 3 10 APMV undetermined serotype APMV-?/pigeon/Otaru/76 L gene

5. Impact, Relevance and Technology Transfer

6 Projects Activities and Outputs

7 Project Productivity

8 Future Work

9 Literature Cited

10 Appendices
10 1 Tables
10 2 Figures
10 3 Publications

I EXECUTIVE SUMMARY

The scientific purpose of the project was to study antigenic and genetic properties of avian paramyxoviruses (APMV) which, apart from a well known Newcastle disease virus (NDV) – the main threat to the poultry all over the world – include 9 antigenic serotypes of rather recently isolated viruses This group of viruses is of high practical importance for international development (both for industrial and less developed countries) and, at the same time, reveals a number of problems of intriguing scientific interest Since the proposal was submitted in 1987 but, by A I D decision, was started only in August 1992, some new developments in the field had to be taken into account. Accordingly, new topics were included and new trends were considered, in particular, substantial emphasis was done to the studies on the genetic level using the RNA sequencing technology The work was carried out according to the six main objectives 1) Comprehensive virological and serological surveillance of domestic and wild birds aiming the isolation of new native APMVs, 2) Comparative studies on the APMV hemagglutinin-neuraminidase (HN) basic properties functional activities and antigenicity studied by means of polyclonal antibodies, 3) Preparation and characterization of monoclonal antibodies (MAB) against envelope proteins of APMV-1 (NDV) and APMV-2 (Yucaipa) viruses, 4) Usage of the MAB panels against the APMV-1 envelope proteins for antigenic characterization of the native NDV isolations, 5) Sequencing of different genes of different APMV serotypes 6) Application of mathematical modeling approach for elucidation of antigenic kinship and phylogenetic relationships between different APMV serotypes In the limits of the objectives 1 & 2, the following topics have been studied (a) antigenic and functional characterization of a collection of APMV- 2 ("Yucaipa-like") viruses isolated in Israel during the epizootics in 1979-81 and then, after about a decade of silence from sporadic isolations from domestic and feral birds, (b) the first isolation and identification in Israel of APMV-4 serotype isolated from wild ducks, (c) APMV isolation and identification in the cases of mixed infection of avian hosts by APMV-1 with APMV-2 and by APMV-1 with APMV-4 viruses (c) antigenic characterization of Enders-Ruckle virus (ERV), an APMV isolated after serial propagation of "normal" chick embryo fibroblasts In the limits of the objectives 3 & 4, the MABs against the envelope protein fraction of APMV-1 and APMV-2 were prepared, in the case of the APMV-1, the specific MAB panels against the HN, fusion (F) and matrix (M) envelope proteins were established and characterized, the non-overlapping groups and antigenic mapping being determined, and the MAB-mediated analysis of the NDV strains isolated in Israel from domestic and feral birds was performed In the limits of the of the objective 5, the direct sequencing and identification of the sequenced pieces of virion RNA corresponding to the APMV different serotypes was achieved Namely the APMV-1 (portions of the HN and L genes), APMV-3a (portions of the P and L genes) APMV-3b (P and M genes' portions), APMV-6 (L gene's portion), APMV-7 (M and HN genes' portions), APMV-9 (HN and L genes' portions), as well as APMV-8 (two not yet identified portions), and APMV-?/pigeon/Otaru (not yet identified portion), Besides, the swine PMV and the ERV of avian origin appeared to be very closely related genetically to the viruses belonging to the avian APMV-3b and to human mumps virus, respectively These are the most important findings having significance for the problems of host specificity to viral infection, viral pathogenicity and persistence The identification of a portion of the HN gene of the APMV-1 isolate from a racing pigeon differing from classic NDV by host specificity is an important fact having significance for potential finding of a specific genomic locus responsible for the of virus host specificity and pathogenicity The sequenced pieces of different APMV genes are a basis for their further comparison on the genomic level The sequence alignment and identification were done via computer connection with EMBO (European Molecular Biology Organization), Heidelberg DataBases Bank The collaboration appeared to be effective Namely during the visit of the Principal Investigator (PI) to the Nairobi University, more than 120 viral agents isolated during the previous year were identified as NDV the virological and ecological surveillance was carried out in lakes Nakuru and Bogoria where 5 NDV were isolated from flamingoes that is of significance from the ecological and epizootiological points of view During the two consecutive visits to Israel with the Kenyan isolates, the CI participated in the experiments on the native viruses' identification, and studied the biochemical, genetic, and immunological methods used in the PI's lab The pace of the research was in accordance with the work plan, although the inclusion of new objectives made it necessary to ask the A I D authorities for an additional year for the project performance

II INTRODUCTION

The proposal was submitted in 1987 and, by some circumstances, the date of August, 1991 was determined by A I D as the starting point of running the project During this period (between the submission of the proposal and the start of the project) new developments in the field, including those published in the world and some data obtained in our lab, had to be taken into account when realizing a technical work plan Virtually, all the objectives, which were in the original proposal, have remained but some new topics were included and new trends were considered This was expressed in certain changes in the research strategy (redistribution of priorities for certain objectives) Namely, much more emphasis was done to the studies on avian paramyxoviruses on the genetic level using sequencing technology Besides, new viruses, which were of especial interest to some tasks of the research, were included into the studies Therefore, certain modifications included into the work plan were realized in the execution of the project and reflected respectively in the present report During the last two years of the project's performance, some new developments appeared to be that was depending on the simultaneously performed work on the collaborative studies with Kazakhstan carried out in the limits of the project also supported by A I D The results obtained in the limits of both the projects were in agreement that permitted to make some general conclusions, especially in the field of viral ecology, concerning the global (intercontinental) spread of paramyxoviruses

III RESEARCH OBJECTIVES

The following objectives have been originally proposed

1 Comparative studies on the basic properties of the APMV HN functional activities

2 Comprehensive comparative studies on direct antigen-antibody binding (ELISA, RIA) and functional (HA and Nase) inhibition cross reaction tests using polyclonal antibodies against all the APMV serotypes

3 Preparation of monoclonal antibodies against HN antigen of some selected APMV serotypes and comprehensive comparative studies on direct antigen-antibody binding (ELISA, RIA) and functional (HA and Nase) inhibition cross reaction tests using the panels of the monoclonal antibodies

4 Probing different APMV serotypes with antibodies to polypeptides expressed from cloned HN gene domains of NDV search for "common", "serotype-specific", "conserved" and "variable" APMV antigenic determinants and their assignment on the genetic level

5 Application of mathematical modeling approach for the elucidation of antigenic kinship and phylogenetic relationships between different APMV serotypes

The objective #5 has been started to be performed since 1987 when the original project proposal was submitted and in general the most of the work was performed before the official approval of the project in 1991 This kind of work, which was possible to be carried out without substantial financial support was done in collaboration with mathematician Prof N Rishe Besides, some new topics were included and new trends were considered. This was expressed in certain changes in the research strategy (redistribution of priorities for certain objectives) Namely, much more emphasis was done to the studies on avian paramyxoviruses on the genetic level using viral gene sequencing technology Besides new viruses, which were of especial interest to some tasks of the research, were included into the studies

In accordance with this, the following objective has been included, namely

6 Sequencing of APMVs' different genes, In the case when full genes are sequenced, determination of open reading frames, initiation and termination points, consensus sequences, and intergenic regions, amino acid sequences and potential glycosylation sites (for hemagglutinin-neuraminidase and fusion glycoproteins)

IV RESEARCH ACCOMPLISHMENTS

1 Viruses Used in the Studies

The whole studies were based on the set of the selected viruses, which included prototype and reference APMV strains as well as local isolates The initial set of viruses which were intended to be studied according to the original proposal, were as follows

List of the APMV serotypes

Serotype		Full designation	Abbreviation
APMV-1		APMV-1/NDV/LaSota	NDV
APMV-2		APMV-2/chicken/California/Yucaipa/56	Yucaipa
APMV-3	variety a	APMV-3a/turkey/Wisconsin/67	Ty/Wisc
	variety b	APMV-3b/parakeet/Netherlands/449/75	Pk/Neth
APMV-4		APMV-4/duck/Hong Kong/D3/75	D3/HK
APMV-6		APMV-6/duck/Hong Kong/D199/77	D199/HK
APMV-7		APMV-7/dove/Tennessee/4/75	Dove/Tn
APMV-8		APMV-8/goose/Delaware/1053/76	Goose/Del
APMV-9		APMV-9/duck/New York/22/80	Duck/NY
APMV-?		APMV-?/pigeon/Otaru/76	Pigeon/Ot

* Serotype APMV-5 (APMV-5/budgerigar/Kunitachi) was not used in the studies since it was not available

The new viruses included into the studies were as follows

1	APMV-1/Racing pigeon/Israel/1831/84	1831
2	APMV-3c/Swine/Nazareth/80	Sw/Naz
3	Enders-Rueckle virus (Avian Mumps-like virus?)	AMuV

The 1st one from the above new viruses was a representative of the strains which were isolated during the panzootics amongst racing pigeons which burst out in Sudan in 198% and spread all over in North Africa, Middle East, including Israel, and Europe This virus falling to the same serotype with Newcastle disease virus - the main threat to the poultry - excited apprehension in the whole world that time as a potential danger to the poultry Therefore, it was of high interest in the limits of the present studies to include this virus into the studies

The 2nd virus was isolated from swine during the surveillance of the Arabic pig farms in Nazareth in 1980 and identified in our la (Unit of Molecular Virology, Division of Avian Diseases) as a paramyxovirus similar to the avian paramyxovirus of the serotype 3, namely it was antigenically very similar to APMV-3 prototype strain APMV-3/parakeet/Netherlands/449/75 (Lipkind et al, 1986) Such close similarity be-

tween an avian and a mammalian viruses could bear a hope to find out some specific sites responsible for the host specificity the comparison of both the viruses at the genetic level (sequence alignment of appropriate gene portions) could reveal these sites

The 3d virus was isolated in 1960 in Tuebingen, Germany, from "normal" chick embryo cell monolayer culture That time this virus was identified as a parainfluenza virus of serotype 2 This virus having been passaged in chick embryonated eggs was forwarded to me by Prof Rudolf Rott in 1990 for a proper antigenic and genetic identification

In addition to the viruses which have been in use in the limits of the project forty three more viruses belonging to the APMV- 2 serotype were employed They included the prototype strain APMV-2/ chicken/California/Yucaipa/56, 11reference strains (one of them has been isolated in Kenya), and 33 local viruses, the majority of which were isolated from turkeys and chickens during 1979-81 epizootics in Israel (see the below list) Since these viruses had been isolated consecutively during about 2 5 years and included feral birds, too, this unique collection was very interesting for the aims of the present project concerning ecological and epizootiological aspects

List of the reference PMV-2 strains and the local PMV-2 viruses isolated in Israel in 1979-81 which were used in the studies

VIRUSES	ABBREVIATIONS
Reference strains	
1 A PMV-2/chicken/California/Yucaipa/56	Yucaipa
2 APMV-2/finch/North Ireland/Bangor/73	Bangor
3 APMV-2/robin/Hiddensee/19/75	Robin
4 APMV-2/cut-throat weaver/Senegal/PLOC-9/76	PLOC
5 APMV-2/mannikin/Japan/Haneda-35/76	Haneda
6 APMV-2/finch/England/347/76	England
7 APMV-2/turkey/Italy/F/77	Italy
8 APMV-2/Sturnus contra/Indonesia/3589/79	Indonesia/3589
9 APMV-2/Sturnus contra/Indonesia/3650/79	Indonesia/3650
10 APMV-2/gadwall/Kenya/80	Kenya
Local isolates	
1 APMV-2/Mallard/Israel, Ramon/4/T/79 1	Mal/Ramon
2 APMV-2/Turkey/Israel, Kfar-Hess/7/T/79 3	Ty/Kfar-Hess/7
3 APMV-2/Turkey/Israel, Kfar-Hess/10A/B/79 3	Ty/Kfar-Hess/10A
4 APMV-2/Turkey/Israel, Kfar-Hess/10B/B/79 3	Ty/Kfar-Hess/10B
5 APMV-2/Turkey/Israel, Burgata/116/B/79 4	Ty/Burgata
6 APMV-2/Turkey/Israel, Beit-Shearim/170/L/79	Ty/Beit-Shearim/170
7 APMV-2/Turkey/Israel, Sde-Yoav/15/T/79 1	Ty/Sde-Yoav/15/T
8 APMV-2/Turkey/Israel, Sde-Yoav/15/C/79 1	Ty/Sde-Yoav/15/C
9 APMV-2/Cattle egret/Israel, Ramat-Gan/9/T/80 9	C egret/Ramat-Gan
10 APMV-2/Turkey/Israel, Beit-Herut/584/B/80 10	Ty/Beit-Herut
11 APMV-2/Chicken/Israel, Mishmeret/589/L/80 10	Ch/Mishmeret/589
12 APMV-2/Turkey/Israel, Neot-Mordehai/593/80 10	Ty/Neot-Mordehai
13 APMV-2/Turkey/Israel, Beit-Shearim/595/80 10	Ty/Beit-Shearim/595
14 APMV-2/Turkey/Israel, Rosh-Tsurim/596/80 10	Ty/Rosh-Tsurim
15 APMV-2/Turkey/Israel, Bear-Tuvia/676/B/80 10	Ty/Bear-Tuvia/676
16 APMV-2/Turkey/Israel, Kfar-Harutz/685/B/80 10	Ty/Kfar-Harutz
17 APMV-2/Turkey/Israel, Nahla/687/B/80 10	Ty/Nahla/687
18 APMV-2/Chicken/Israel, Hatsav/734/B/80 11	Ch/Hatsav/734
19 APMV-2/Turkey/Israel, Bear-Tuvia/752/B/80 11	Ty/Bear-Tuvia/752
20 APMV-2/Chicken/Israel, Mishmeret/803/K/80 11	Ch/Mishmeret/803

21 APMV-2/Turkey/Israel, Shalva/810/B/80/11	Ty/Shalva
22 APMV-2/Turkey/Israel, Nahla/812/B/80 11	Ty/Nahla/812
23 APMV-2/Turkey/Israel, Sde-Moshe/813/B/80 11	Ty/Sde-Moshe
24 APMV-2/Coot/Israel, Shluhot/868/S/80 12	Coot/Shluhot
25 APMV-2/Chicken/Israel, Petahia/817/B/81 1	Ch/Petahia
26 APMV-2/Turkey/Israel, Ein-Tsurim/818/B/81 1	Ty/Ein-Tsurim
27 APMV-2/Turkey/Israel, Tsora/830/B/81 1	Ty/Tsora
28 APMV-2/Turkey/Israel, Talmi-Ehiel/837/B/81 1	Ty/Talmi-Ehiel
29 APMV-2/Turkey/Israel, Beit-Yitzhak/846/C/81 1	Ty/Beit-Yitzhak
30 APMV-2/Turkey/Israel, Hatsav/4/T/81 1	Ty/Hatsav/4/T
31 APMV-2/Turkey/Israel, Hatsav/6/T/81 3	Ty/Hatsav/6/T
32 APMV-2/Coot/Israel, Beit-Alfa/5/T/81 3	Coot/Beit-Alfa
33 APMV-2/Turkey/Israel, Hatsav/8/T/81 6	Ty/Hatsav/8/T

After a decade of "silence" (no APMV-2 ["Yucaipa-like'] virus was isolated in spite of the wide surveillance) some new isolations appeared to be picked up both from domestic and feral birds Accordingly the following viruses were involved into the studies

List of the APMV-2 strains isolated in 1994

VIRUSES	ABBREVIATIONS
1 APMV-2/Chicken/Israel, Hadera/1545/94 3	Ch/Hadera/1545
2 APMV-2/Chicken/Israel, Hadera/2058/94 3	Ch/Hadera/2058
3 APMV-2/Turkey/Israel, Hatsav/2470/94 4	Ty/Hatsav/2470
4 APMV-2/Turkey/Israel, Hatsav/1441/94 5	Ty/Hatsav/1441
5 APMV-2/Turkey/Israel, Hatsav/1442/94 5	Ty/Hatsav/1442
6 APMV-2/Turkey/Israel, Hatsav/822/94 5	Ty/Hatsav/822
7 APMV-2/Turkey/Israel, Hatsav/2986/94 5	Ty/Hatsav/2986
8 APMV-2/Turkey/Israel, Hatsav/2264/94 5	Ty/Hatsav/2264
9 APMV-2/Turkey/Israel, Ramot/1852/94 5	Ty/Ramot/1852
10 APMV-2/Turkey/Israel, Ramot/513/94 5	Ty/Ramot/513
11 APMV-2/Turkey/Israel, Ramot/1750/94 5	Ty/Ramot/1750
12 APMV-2/Turkey/Israel, Ramot/1956/94 5	Ty/Ramot/1956
13 APMV-2/Turkey/Israel, Ramot/37/94 5	Ty/Ramot/37
14 APMV-2/Turkey/Israel Ramot/2369/94 5	Ty/Ramot/2369
15 APMV-2/Turkey/Israel, Ramot/40118/94 5	Ty/Ramot/40118
16 APMV-2/Turkey/Israel, Herut/2161/94 7	Ty/Herut/2161
17 APMV-2/Duck/Israel, Herut/1344/94 7	Dk/Herut/1344
18 APMV-2/Duck/Israel Herut/1750/94 7	Dk/Herut/1750
19 APMV-2/Imperial sandgrouse, T-A Zoo/3191/94 5	Imp sgr/Zoo

2 Basic Properties of the APMV Hemagglutinin-Neuraminidase Glycoprotein

2 1 Thermostability of hemagglutinin-neuraminidase (HN) glycoprotein of the APMV prototype strains

In the limits of this objective, the functional thermostability of hemagglutinin-neuraminidase (HN) glycoprotein was examined Hemagglutinating (HA) and neuraminidase (NA) activities were determined before and after the heating the virus preparation at 56° C for 1 hr Since the thermostability is very often °used as a convenient marker it was valuable to use in the studies the simplest virus preparation which is the ordinary virus-containing allantoic fluid. Therefore, the thermostability was determined in both allantoic virus and purified virus preparations in order to elucidate the possible influence of components of allantoic fluid on the thermostability The results are presented in Tab 1

2 2 Antigenic characterization of Enders-Ruckle virus (ERV), an APMV isolated after serial propagation of "normal" chick embryo fibroblasts

This virus was isolated in 1960 in Tuebingen Germany from "normal" chick embryo cell monolayer culture (Enders-Ruckle 1960) That time this virus was identified as a parainfluenza virus of serotype 2 Antibodies against this virus were found in many poultry farms in Germany using the same test (Wagner & Koesters 1965) However this identification was done in incomplete way without performing a proper cross-reaction test. Therefore, the virus was forwarded to me by Prof Rudolf Rott in 1990 for a proper antigenic and genetic identification

The antigenic characterization of the ERV was carried out first by means of hemagglutination inhibition cross-reaction test using polyclonal antisera and then by means of ELISA using monoclonal antibodies (MAB) against hemagglutinin-neuraminidase (HN) of human parainfluenza virus serotype 2 (PIV-2) which were kindly gifted by Dr Claes Orvell

The experiments with polyclonal antisera have shown that the ERV is much closer antigenically to the group NDV-mumps virus than to the PIV- 3 virus The experiments with MABs against PIV- 2 have shown that none of 12 MABs against HN glycoprotein of PIV- 2 react with ERV

Then taking into account the closeness of the ERV to mumps virus it was decided to employ the panels of MABs against mumps virus (strain SBL) which also were provided by Dr C Orvell in ELISA with ERV It turned out to be that 50 MABs against five viral proteins [HN, fusion (F) matrix (M), nucleoprotein (NP) and phosphoprotein (P)] reacted with ERV (Tab 2)

In order to elucidate whether the ERV (or ERV-like viruses) is spread amongst the domestic bird population in Israel an extensive serological surveillance has been carried out. The methodical approach consisting in blocking of binding of certain anti-mumps MABs to ERV antigens in ELISA test. The results have shown that about 5-15% of chicken field sera collected in several poultry farms in Israel were positive in this tests This indicates the presence in the field sera of antibodies that can bind to mumps virus (Tab 3)

Thus the above results have indicated very close relatedness of the ERV to the human mumps virus Since this was an extravagant conclusion genetic characterization of the ERV using sequencing technology was undertaken (see the paragraph 3a)

2 3 Functional properties of the HN of APMV-2 ("Yucaipa-like") viruses

These properties included some the HN thermostability and enzymatic activity and electrophoretic mobility of all the viral structural properties of APMV-2

2 3 1 Property of elution from red blood cells

During the preliminary studies, it was noticed that several isolates showed rapid elution after HI tests had been done More systematic evaluation of this phenomenon revealed considerable variation among the strains and isolates tested (Tab 4) Of the reference strains tested, only Haneda and Bangor showed full elution which occurred within 4 hours others showed either no elution after 24 hours or only partial elution at this time Thirty-three field isolates were also tested and these showed considerable heterogeneity in terms of elution (Tab 4) Most of the strains (23) were partial or moderate eluters, but five isolates were non-eluters and five classed as fast eluters

2 3 2 Electrophoretic mobility of APMV-2 viral structural proteins

Purified preparations of 16 of the Israeli isolates were subjected to SDS-PAGE in comparison with the Yucaipa and NDV/Ulster viruses (Fig 1, parts 1, 2, 3) The overall polypeptides patterns of the isolates were similar to each other consisting of the same number and approximate size of polypeptides (the lines between the L and HN proteins in the Fig 1, part 1, are probably impurities) but differed from the reference Yucaipa virus in migration rate of most the polypeptides However, more detailed examination revealed slight differences amongst the isolates which were expressed in the position of the F_0 and the M proteins and the band density of the HN protein. By these indications, all the studied viruses can be distributed into two groups The group A included viruses whose F_0 glycoprotein migrated faster, the M protein migrated slower and the density of the HN protein band was significantly lower as compared to the respective values of the viruses belonging to the group B (Fig 1, parts 1, 2, 3) The difference in the HN band density is most clearly demonstrated in the case of Ty/Neot-Mordehai (Fig 1, part 1), Ty/Beit-Shearim (Fig 1, part 2), and Mal/Ramon (Fig 1, part 3) viruses where the above low density of the HN protein is combined with the higher density of the NP and M bands (higher if compared with the respective bands of the viruses belonging to the group B) The group A included the viruses Ty/Kfar-Hess/10A, Ty/Kfar-Harutz, Ty/Neot-Mordehai,Coot/Shluhot (Fig 1, part 1), Ty/Sde-Yoav/15/T, Ty/Beit-Shearim/170, Ty/Bear-Tuvia/752 (Fig part 2), Mal/Ramon and Ch/Mishmeret (Fig 1, part 3) The group B included the viruses Ty/Shalva C egret/Ramat-Gan, (Fig 1, part 1), Ty/Sde-Yoav/C/15, Ty/Bear-Tuvia/676, Ch/Hatsav (Fig 1 part 2), Ty/Nahla/687 and Coot/Beit-Alfa (Fig 1, part 3) The full list of the reference and Israeli "Yucaipa-like" viruses is presented in the previous report.

2 4 Antigenic heterogeneity by HN antigen amongst the APMV-2 ("Yucaipa-like") viruses

A large number of "Yucaipa-like" viruses have been isolated up to eighties (Fleury and Alexander, 1979, Fleury et al , 1982) Therefore, it was considered important to investigate the antigenic relationships between these "reference" viruses to enable assessment of the significance of differences that may be detected within the Israeli isolates Consequently, at first, the antigenic differences amongst the reference strains have been studied, that being a basis for the comparison of the rich collection of the APMV-2 isolates from the local reservoir This gave the basis for the conclusions of high ecological, epizootiological and phylogenetic importance

2 4 1 Antigenic heterogeneity amongst the APMV-2 reference strains hemagglutination inhibition cross reaction tests with reference APMV-2 viruses

Ten reference and two Israeli APMV- 2 viruses and antisera against four of the reference (Yucaipa, Robin, PLOC, and Haneda) and the above two Israeli strains which included the first isolate made at the beginning of the epizootic in April 1(71 (Ty/Kfar-Hess/10B) and one isolated at the peak of the epizootic in November 1980 (Ch/Hatsav) were used in these experiments

The cross reactivity was expressed as difference between the homologous and heterologous hemagglutination inhibition (HI) titers expressed in dual log It can be seen (Tab 5), that the two selected Israeli isolates did not differ from each other by the criterion of cross reactivity all the reference viruses were inhibited at high levels by each serum and the degree of the cross reactivity varied considerably so that amongst these strains no one was similar to each other For example, for Yucaipa, Robin, PLOC and Haneda viruses whose antisera were also used in the tests and, hence, the two-side cross reactivity could be determined and quantitated, the pairwise comparison showed no similarity between the four strains In the case of two pairs, Yucaipa-Robin and PLOC-Haneda, one-sided asymmetric cross reactivity was observed when anti-Yucaipa serum against Robin and anti-PLOC antiserum against Haneda were used there was no difference between homologous and heterologous HI titers (*d* is close to zero) while when anti-Robin serum against Yucaipa and anti-Haneda serum against PLOC were used then the difference *d* was more than 3 log in the former case and more than 3 log in the latter one (Tab 5) In the case of Yucaipa-Haneda pair, there was two-sided asymmetric cross reactivity In the other pairs related to these four viruses, there was symmetric cross reactivity, the *d*, indication being in the range between 1 and 2 $\log_2$ These four strains also differed in the pattern of their cross reactivity with the Israeli isolates For example, PLOC showed no difference with Ty/Kfar-Hess/10B virus and low one-side asymmetric cross reactivity with the Ch/Hatsav isolate Robin showed one-side asymmetric cross reactivity with both the native isolates Yucaipa showed difference with both the native strains displaying two-sided (Ty/Kfar-Hess/10B) and one-sided (Ch/Hatsav) asymmetric cross reactivity, Haneda showed symmetric (Kfar-Hess/10B) and one-side asymmetric (Ch/Hatsav) cross reactivity (Tab 5)

For the other six APMV-2 reference viruses against which there was no antiserum and, hence, two-side cross reactivities could not be determined, a comparison could be made only with respect to their HI titres with antisera against the four reference viruses (Tab 5) According to this criterion, some regularitiescan be noticed Namely, all the viruses show similar relationship to Robin (line 2), the difference between homologous and heterologous HI titers (*d*) ranging between 1 4 and 3 4 $\log_2$ The same pattern was seen with Haneda with *d*, ranging between 1 and 2 5 $\log_2$ (line 4), the strain Indonesia/3650 being the only isolate not differing from the homologous virus At the same time, all the viruses showed antigenic similarity with the PLOC virus, *d* being statistically non-significant (close to zero) Yucaipa virus showed similarity with England, Italy Indonesia/3589 and Indonesia/3650 (no significant difference of *d* from zero) while it differed from Bangor and Kenya Both the antisera against the two Israeli viruses showed similarity with the viruses Italy, Indonesia/3589 and Kenya with the other reference strains there were different combinations Ty/Kfar-Hess/10B did not differ from the reference strains, except, Bangor· Ch/Hatsav showed difference also with England and Indonesia/3650 strains

Thus, summarizing the above data, it can be concluded that viruses Robin and Haneda are similar by two-way cross reactivity while the viruses Bangor and Kenya, on one hand, and Italy and Indonesia/ 3589 on the other hand, are similar according to the less strong criterion of the spectrum of antigenic relationships (one-side cross reactivity) with the four viruses (Yucaipa, Robin, PLOC and Haneda) Bangor and Kenya by the same criterion are similar to the PLOC virus and show significant difference from Yucaipa, Robin and Haneda

2 4 2 Antigenic heterogeneity amongst the APMV-2 viruses isolated in Israel

These studies offered a unique opportunity to detect antigenic variations in detailed dynamics and, thus reveal evolutionary pathways, this being one of the objectives of the research. During the reported period, the studies concerning the antigenicity of above viruses were finished and supplemented with the studies on characterization of two other properties of these viruses, namely, the electrophoretic mobility of their proteins and another functional property – the rate of elution of the viral particles pre-adsorbed upon the red blood cell (RBC) surface The latter property is a complex one depending on both viral enzymatic and receptor-binding activities

2.4 2.1 Isolation and identification of the APMV-2 viruses isolated in Israel in 1979-81

Up to 1979 no avian paramyxovirus other than NDV had been isolated in Israel. Systematic surveillance of wild and domestic birds for influenza viruses was begun in 1979 and as a consequence of these studies a PMV- 2was isolated in January 1971 from mallard ducks (*Anas platyrhynchos*) migrating into Israel (Lipkind et al 1982b) During the winter of 1978-71 a series of respiratory disease problems occurred on turkey farms in Israel and had appeared to spread The disease was characterized by signs ranging from mild rales to severe respiratory distress including conjunctivitis, nasal discharge, sinusitis and pneumonia, egg production was severely affected in breeder flocks Morbidity usually approached 100% while mortality varied from 5% to 90% (Lipkind et al 1979) In March 1971 several hemagglutinating (HA) agents isolated from outbreaks of the disease were identified as avian PMV of the PMV- 2 serotype Since that time investigation of the disease in turkeys and surveillance of other birds has resulted in the isolation of more than 70 PMV- 2 viruses from all parts of Israel (Lipkind et al 1982c) However experimental infection of turkeys by the isolated Yucaipa virus did not caused clear clinical symptoms unless the infection by the Yucaipa virus was combined with some bacterial or mycoplasma agents which were also isolated that time from affected turkey flocks (Heller et al 1984)

The majority of the isolates were obtained from turkeys but isolations have also been made from domestic fowl (Lipkind et al 1982c) coots (*Fulica atra*) [Lipkind et al 1982a] and cattle egret (*Ardeola ibis*) [Lipkind et al 1981]

Such a rich collection of the viruses isolated consecutively in the same geographic area from domesticated and feral birds offered a unique opportunity to detect antigenic variations in detailed dynamics and thus reveal evolutionary pathways which is one of the objectives of the research.

Thirty-three viruses of PMV- 2 serotype isolated in Israel from domestic and wild birds during epizootic of a respiratory disease in 1979-1981 were studied comprehensively in comparison with a set of reference PMV- 2 viruses using cross reaction hemagglutination inhibition tests The results demonstrated significant heterogeneity amongst the local isolates There was no significant correlation between the differences found and chronology of isolation of the viruses For more definitive conclusions about antigenic heterogeneity of the Yucaipa-like isolates in association with ecological epizootiological and phylogenetic aspects some additional viral properties are to be studied

2 4 2.2 Antigenic heterogeneity amongst the APMV-2 viruses isolated in Israel in 1979-81 hemagglutination inhibition cross reaction tests

The results of HI tests with the field isolates are distributed according to the year of isolation within 1979-81 in Tables 6, 7 and 8, respectively

Although there was a considerable heterogeneity among the field strains, certain regularities can be noticed

With Yucaipa antiserum only 6 of 33 strains showed low, although significant, difference $d > 0$, from the homologous titres, of these 5 strains showed differences $d > 1$ Mal/Ramon, Ty/Kfar-Hess/10B Ty/Burgata (Tab 6), Ty/Nahla/687 (Tab 7) and Ch/Petahia (Tab 8) For the rest of the strains d fluctuated around zero

For Robin and Haneda antisera, the values of the d demonstrated a generally higher difference In the case of anti-Robin antiserum, 12 isolates showed high difference ($2\ 0 < d < 3\ 0$), the Ch/Nahla strain having shown d, $= 3\ 37 \pm 1\ 08$, 11 isolates showed the moderate difference ($1\ 0 < d < 2\ 0$) and the rest (10 isolates) showed low or no difference In the case of the anti-Haneda antiserum, 5 isolates showed high difference two of them having shown $d > 3\ 0$, 16 isolates showed moderate difference and the rest (10 isolates) – low or no difference

In the case of the anti-PLOC antiserum, 10 isolates showed moderate difference, one isolate showed *d* = 3 21± 0 32 and the rest (22 isolates) - low or no difference

This evident heterogeneity amongst the Israeli strains revealed by the cross reactivity with reference antisera was in contrast to the results manifested by HI tests with two antisera against two selected Israeli viruses, in which case the values of d, showed no significant difference from zero The only exceptions were Ch/Petahia and Ty/Ein-Tsurim strains which showed significant difference from the homologous viruses (d > 1) with both the antisera and Coot/Beit-Alfa and Ty/Kfar-Hess/7 strains which showed significant difference with one of the antisera.

In some cases the *d* indication had negative (statistically significant) values which meant that the heterologous HI titer was higher that the homologous As such a phenomenon was observed usually with all or most of the six reference antisera reacted with any virus, this might reflect a particular sensitivity of the viral antigen to antibodies, which has been known as the phenomenon of "avidity" The isolates Ty/Nahla/812 and Ty/Sde-Moshe (Tab 8) are the best expressions of such avidity

2.4 2.3 New isolations of APMV 2 ("Yucaipa like") viruses in Israel from domestic and feral birds after decade of silence

During the execution of the project, in the period of March - July, 1994, there were cases of mild respiratory disease in turkey and chicken farms with low mortality Cloacal and tracheal swabs taken from sick birds were stored frozen till July 1994 and then propagated through chick embryonated eggs The isolated hemagglutinating agents were identified as APMV-2 (prototype strain - APMV-2 /Chicken/Yucaipa/California/56) on the basis of hemagglutination inhibition and neuraminidase inhibition tests Most of the isolations were related to the cases occurred in May, 1994 In July, 1994, a similar syndrome was observed in a duck farm from where the APMV-2 ("Yucaipa-like") viruses were also isolated During the same period, the Yucaipa-like virus was isolated from an imperial sandgrouse (Pterocles orientalis) kept in Zoo of Tel Aviv University The isolates are listed in the Tab 9

The point of importance was comparison of the above-described APMV-2 viruses (isolated in 1994) with the APMV-2 viruses isolated during the epizootic 1979-81 In the present report, both the groups of viruses were compared by the thermostability of their hemaggutinins and by the rate of elution from red blood cells

The results on the thermostability are presented at the Tab 10 & Tab 11 It can be seen that the ratio of the thermostable strains is significantly higher amongst the "new" (1994) isolates as compared to the "old' (1979-81) isolates (404 against 114 respectively)

The elution rate of the "old" strains (Tab 12) was compared with the corresponding results concerning the new strains (Tab 12) The comparison has shown that the ratio of non-eluters is significantly higher amongst the "new" isolates as compared to the "old" ones (60% against 15%, respectively)

2 4 3 Discussion and conclusions Ecological and evolutionary enigmas endemic source or "import"?

The antigenic interrelationships which have been displayed graphically (Fig 2), show close elatedness, almost identity (in the limits of serological criteria), between the two Israeli isolates and the PLOC virus In general, the PLOC virus has the most close relatedness with the other members of the graph from 5 possible interconnections, two designate close relatedness, two - moderate, and one - low The Robin virus has moderate cross reaction with 3 viruses and low with two others Haneda virus displays two moderate and three low relations and Yucaipa virus shows only one moderate (with PLOC) and four low relationships This picture is compatible with the theories concerning the presence of the "common" and "serotype-specific portions on the genomic and, correspondingly, the antigenic level (Lipkind & Shihmanter, 1986) and the notions of "con-served" and "variable" regions (Nishikawa et al , 1983) and "strain-specific" epitope on the NDV HN molecule recognized by monoclonal antibodies (Iorio & Bratt, 1(84) In accordance

with this, one can speculate that amongst the PMV- 3viruses used in the studies the PLOC virus has the largest "common" portion while the Yucaipa virus has the smallest

The close relatedness of the Israeli isolates to the PLOC virus is supported by the data on the PLOC's relationships with all the other Israeli isolates (Tab 6, 7, 8) On the basis of the above data, it can be suggested that the PLOC virus is the ancestor of the Israeli isolates or both have the common ancestor from whom they have not drifted far away in a fan-like direction. From the ecological point of view, the relationship of the Israeli isolates with PLOC virus would be expected since Israel is on the migratory route of passerine traveling from Europe to West Africa

However, the main objective of the studies was an attempt to find out whether the antigenic drift could occur during the epizootics and could be revealed by analyzing antigenic heterogeneity among the consecutively isolated viruses The heterogeneity was demonstrated by different parameters

First, the detailed comparison of two Israeli isolates which were selected as "native reference" viruses - Ty/Kfar-Hess/10B and Ch/Hatsav - with the reference APMV- 2 strains revealed difference between them although the direct comparison of both did not show significant difference in cross reactivity However the two viruses clearly differed from each other by their cross reactivity with the other PMV-2 Besides, there was no parallelism between the two viruses in their cross reactivity with the PLOC, Haneda, and Indonesia/3650 viruses Such a phenomenon when there is no significant antigenic difference between two viruses but they differ by the spectrum of their cross reactivity with some other viruses has been described earlier (Lipkind & Shihmanter, 1986, Lipkind et al , 1986)

It is not possible to arrange the isolates in an order displaying consecutive accumulation of changes along with the time axis, which could demonstrate an antigenic drift According to the dynamics of isolations of different antigenic variants, it seems that the isolates did not derive from a certain ancestral strain as a result of consecutive changes but there was a ***co-circulation of a number of APMV- 2 variants which appeared at the very beginning of the epizootic.***

This suggestion is supported by the results on the hemagglutination-elution pattern (Tab 4) On one hand, there is a certain connection between antigenicity and hemagglutination-elution pattern, namely, four from the five non-eluter viruses are those belonging to the group 4 from which three were isolated in 1979 But this is the only one clear correlation on the background of flat randomness However, to some extent, a chronological relationship can be seen in the groupings of strains on elution ability in that the earlier isolates were non-eluters and fast eluters appeared in 1980 (Tab 4) However, some strains isolated in 1980 and even in 1981 were moderate eluters Moreover it was noticed that during passaging the elution properties of certain strains were changing On the basis of these data, we suggest that within the population of the APMV- 2 viruses there should be two sorts of virions in respect to their elution-hemagglutination property - "non-eluter" and "fast eluter" particles In accordance with this, all the spectrum of the found differences should be due to different proportions of "non-eluter" and "fast eluter" particles in the heterogeneous viral populations of the corresponding strains This is one more indication that during the studied period (1979-1981) in the avian population in Israel there was co-circulation of different variants, differing at least, by antigenicity and elution-hemagglutination properties

The distribution of the isolates by the PAGE pattern of the viral protein migration rate also has revealed no correlation with the previously described grouping of the isolates by both antigenicity and elution-hemagglutination pattern Namely, if comparing the grouping by PAGE pattern with that by antigenicity it can be seen that both the groups A and B (PAGE pattern) include the viruses belonging to different groups qualified by antigenicity and some viruses belonging to the same groups qualified by antigenicity may belong to different groups qualified by PAGE The same situation can be seen if comparing the grouping by PAGE with that by elution-hemagglutination pattern. Namely, most of the viruses (5 from 9) belonging to the group A are moderate eluters (Ty/Kfar/Hess/10A, Ty/Neot-Mordehai, Coot/Shluhot, Ty/Bear-Tuvia/752 and Mal/Ramon), but two viruses are partial eluters (Ty/Kfar-Harutz and Ty/Beit-Shearim), one is a non-eluter (Ty/Sde-Yoav/15/T) and one is a fast-eluter (Ch/Mishmeret/58), the group B includes Ty/Nahla/687 which is a non-eluter, on one hand, and C egret/ Ramat-Gan which is a fast eluter on the other hand (Tab 4 and Fig 1, parts 1, 2, 3)

Thus, the general conclusion from the studies is that the PMV- 2 viruses isolated in Israel during the period 1979-81 demonstrate heterogeneity by antigenicity of the HN glycoprotein, elution-hemagglutination pattern and PAGE protein migration pattern with no evident correlation between the three indications, i e the grouping of the isolates by any of the above criteria does not coincide with each other Together with this, there is no evident correlation between the above parameters and the chronology of the virus isolations This is a basis for suggestion that during the epizootic there was a co-circulation of different variants of the PMV-2 virus varying by at least three different properties which were not necessarily combined The most striking fact in this respect concerns the viruses Ty/Sde-Yoav/15/T and Ty/Sde-Yoav/15/C which were isolated from tracheal and cloacal swabs, respectively, taken from the same individual turkey and which differed by antigenicity (Tab 6), elution-hemagglutination (Tab 4) and PAGE protein migration (Fig 1) patterns In addition to these facts, there have been some observations on changing elution-hemagglutination pattern along with the consecutive virus passaging

In connection with this, the intriguing question is whether such wide heterogeneity of the Israeli APMV- 2 isolates is a result of remarkable variability of a hypothetical initially homogeneous virus which would cause the epizootic and be the ancestor of all the varying isolates, or the causative viral factor was heterogeneous (consisted of heterogeneous virion population) from the very beginning of the epizootic The above-described experimental evidence demonstrating the heterogeneity among the early isolates supports the latter hypothesis This, in turn, leads to two alter-native suggestions

1 The APMV- 2 virus had been circulating in the local avian reservoir long before the beginning of the described outbreak, which means that it, was enzootic rather than epizootic Enzootic origin of the local APMV- 2 isolations would explain evolutionary antigenic and other biological changes, which led to accumulation of the corresponding variants In such a case there should be a certain "provocative" factor which would "wake up" the "sleeping" enzootic PMV- 2 virus Turkey rhinotracheitis (TRT) pneumovirus would have been such a provocative factor the disease was diagnosed by clinical syndrome in 1978, the viral agents were isolated in 1980 and were identified as TRT in 1987 (Weisman et al , 1989)

2 The APMV- 2 virus was brought into the local avian reservoir from "abroad" (real epizootic) probably via migrating birds but the "imported" virus was already heterogeneous

The choice between the two alternatives is due to the existing ecological evidence According to it, dozens of HA agents identified either as influenza viruses or APMVs were isolated before and after the epizootic with no APMV- 2 among them although massive surveillance and swabbing of domestic and feral birds began before the epizootic and went on in the same intensity after it This is not compatible with the first alternative while fully agree with the second one Since the Mal/Ramon virus has been the earliest Israeli PMV- 2 isolation preceding the epizootic in poultry, it is tempting to speculate about inter-species transfer of the PMV-2 virus from wild migrating to local domestic birds However, in such a case, the Mal/Ramon virus was not a pure genetic ancestor but was initially heterogeneous

The hypothesis about the initial heterogeneity is compatible with the views about rapid evolution of RNA viruses (Holland et al , 1982) and micro-heterogeneity of RNA genomes (Steinhouer & Holland, 1987) based on the ***quasi-species concept***, (Eigen et al , 1(81) According to these views, the multiple mutational changes are not evolved sequentially but are diverged from a common ancestor and, hence, together with the main gene sequence, there is a "quasi-species" mutant distribution (Eigen and Schuster 1979, Eigen et al , 1981) These rapidly mutating subpopulations can remain remarkably stable under certain conditions of replication but when the conditions are suddenly changed, the equilibrium between the virus main population and multiple subpopulations becomes disturbed resulting in selection of a certain subpopulation(s) which become(s) the main one(s) This has an image of extremely rapid evolution and an acute outbreak of a virus disease is an effective factor for such rapidity

The above considerations are relevant not only to the heterogeneity of the local PMV-2 isolates but to the reference PMV-2 viruses as well since they also have shown a wide heterogeneity by the above three indications

Thus, the results demonstrated considerable heterogeneity amongst the local isolates by the above three criteria which were not correlated with each other There was no significant correlation between the differences found and chronology of isolation of the viruses The results indicated that there may have been simultaneous co-circulation of different PMV-2 strains in the local avian reservoir at the beginning of the epizootic rather than temporal accumulation of variants as a result of antigenic drift of an "ancestor" virus which caused the initial infection

The isolation of APMV-2 in Israel after decade of silence raises the question whether there was appearance of a new virus or "awakening" of the "old" virus which had been preserved somehow in the natural reservoir In this respect, the comparison of the 'new" and "old" viruses is of high importance

On one side, it has been shown (Lipkind at al , 1995) that there may have been simultaneous co-circulation of different strains at the beginning of the epizootic rather than temporal accumulation of variants as a result of antigenic drift of an "ancestor" virus which caused the initial infection. The same situation seems to have place in the case of the "new" isolates Also amongst the isolates there was a combination of the thermostable and thermolabile strains, on one hand, and fast eluting and non-eluting strains, on the other A typical example of the initial heterogeneity was the occurrence of two duck APMV-2 isolates picked up in the same place at the same time and sharply differed by the thermostability and the rate of elution. It is also of interest that these two properties were not unequivocally connected namely both non-eluting and fast eluting viruses could turn out to be either thermostable or thermolabile

On the other side, a certain difference between the "old" and the "new" populations could be suspected, namely, both the populations, being heterogeneous, significantly differed by the ratio of the strains characterized by either thermostability or the rate of elution amongst the whole populations

Therefore, a point of the high importance is the antigenic and genetic comparison of the newly isolated "Yucaipa-like" virus strains with those isolated during the epizootics 1979-81 Since then, there was no isolation of the "Yucaipa-like" virus till 1994, although massive continuous surveillance was carried out and plenty of various other viruses have been isolated including other APMV serotypes and influenza viruses The above-discussed problem of ecological and epizootiological importance about whether Israel is an endemic source of Yucaipa virus, or this virus is "imported" via migrating birds would get another source for elucidation In accordance with this, the antigenic, genetic and phylogenetic studies on comparison of "old" and "new" "Yucaipa-like" viruses isolated in Israel together with reference strains, are now in progress

2 5 The first isolation of APMV serotype 4 (APMV-4) in Israel antigenic peculiarities of the local isolates

Cloacal and tracheal swabs taken from 36 wild mallard ducks shot by hunters in Northern part of Hoolah Valley (Upper Galilee) were propagated through chick embryonated eggs One hemagglutinating (HA) agents from tracheal and three from cloacal swabs were isolated All the isolates possessed neuraminidase activity proving their belonging to either ortho- or paramyxovirus family Double immunodiffusion test with antiserum against influenza virus nucleoprotein antigen gave negative result hemagglutination inhibition (HI) test with reference anti-sera against all the nine serotypes of avian paramyxoviruses (APMV) showed that one of the isolates was Newcastle disease virus (NDV) while the three others showed positive results with antiserum against prototype strain (APMV-4/duck/3/Hong Kong/75) of the serotype 4 of avian paramyxoviruses (Tab 13) However when cross reaction HI test was performed using chick antisera prepared against the isolates the cross reactivity between the isolates and the prototype strains was found to be asymmetric Besides the isolates as compared to the prototype virus showed more closed antigenic relatedness to NDV (Tab 14) This is the first isolation of APMV of serotype 4 in Israel

2 6 Isolation of two different APMV serotypes in the cases of mixed infection of domestic and feral birds in Israel

2 6 1 Mixed infection by APMV-1 and APMV-2 viruses

Amongst the Yucaipa-like viruses isolated in May 1994, there was one isolated from turkey (designated as APMV-2/turkey/Herut/24-70/94) which was inhibited also by anti-NDV antiserum Besides, there were two more Yucaipa-like isolates (APMV-2/chicken/Hadera/28-83/94 and APMV-2/turkey/ Hatsav/29-86/94) which became be inhibited by the anti-NDV antiserum after two passages through embryonated eggs A mixed infection by APMV-2 (Yucaipa-like virus) together with APMV-1 (NDV) was suspected This was proven by the experiment on treatment of the isolate by either anti-NDV or anti-Yucaipa virus antisera followed by the propagation of both the portions of the treated isolate through embryonated eggs (Tab 15) After incubation, the yielded viruses were identified as NDV (from the portion treated with the anti-Yucaipa antiserum) and as Yucaipa virus (from the portion treated with anti-NDV anti-serum) Such separate isolation of APMVs from single isolates proved mixed infection of poultry farms by two viruses Conversion of isolates initially identified as APMV-2 virus into those identified as APMV-1 (NDV) as a result of the propagation can be explained by the prevalence of the NDV in the multiplication rate in the embryonated eggs as compared to APMV-2

2 6 2 Mixed infection by APMV-1 and APMV-4 viruses

One of the hemagglutinating (HA) agents isolated from wild mallards (Anas plathyrrhynchos) in February 1994 caught in village Beerim (Negev desert) showed negative result in HA inhibition (HI) test with the reference anti-NDV antiserum. Then, the wide HI test using reference antisera against all the APMV serotypes was performed The results have shown inhibition of the isolate by antiserum against APMV-4 serotype (the prototype strain APMV-4/duck/Hong Kong/D3/75) Consequently, the isolate was identified as APMV-4 and designated APMV-4/mallard/Israel-Beerim/51-152/94 For a proper identification of the isolate by HI and NI inhibition cross reaction tests, chicken antiserum against the isolate started to be prepared using the allantoic virus after the 3d passage of the initial isolate However, the chickens inoculated by this virus preparation fell sick displaying neurological symptoms characteristic for Newcastle disease Then, the virus used for the immunization was checked in HI test in parallel to the original isolate (before the passaging) The results have shown that the original virus still had the serological properties of the APMV-4 serotype while the isolate after two passages acquired the /property to be inhibited by the anti-NDV (APMV-1 serotype) Then the mixed infection by APMV-1 and APMV-4 was suspected In order to prove it, the treatment of the initial isolate by either anti-NDV or anti-APMV-4 antisera followed by the propagation of both the portions of the treated isolate through embryonated eggs was carried out After incubation, the virus yielded from the portion treated with the anti- APMV-4 antiserum was identified as NDV and the virus yielded from the portion treated with anti-NDV anti-serum was identified as APMV-4 (Tab 15) The "conversion" of the isolate initially identified as APMV-4 virus into that identified as APMV-1 (NDV) in the process of propagation can be explained by the prevalence of the NDV in the multiplication rate in the embryonated eggs as compared to the APMV-4

Discussion and conclusions to 2 6 The fact of the mixed infection of birds by two APMV serotype may occur to be of high epizootiological and pathogenic importance Double APMV infection in poultry may be followed by the interference phenomena resulting in either facilitation of or hindrance to of infection and clinical syndrome development. The occurrence of double infection in poultry farms where anti-NDV vaccination is a loyal routine has serious consequences One of the questions of the immediate practical importance is that whether the NDV component in the mixed infection originates from the vaccine strain. tasks If this is not the case and the NDV component has nothing to do with the vaccine strain there is another problem how NDV overcomes the vaccination. In this respect the role of the accompanying second APMV may be decisive and become an obstacle for successful vaccination. As far as the double infection in the wild mallard is concerned, the NDV vaccination is out of question, the NDV component of the isolate is evidently virulent and the epizootiological importance of these findings is to be elucidated

2 7 Virological Surveillance and Serological Identification of the Hemagglutinating Agents Isolated in Kenya

2 7 1 Field surveillance of avian population poultry and feral birds

The work described here is a part of the studies led and carried out in Kenya by Prof Philip N Nyaga and his team The initial objective of this part of the studies to be performed in Kenya in the limits of the CDR project was as wide as possible surveillance of domestic and wild birds in the country in attempt to isolate APMVs Only then it would be possible to carry out comparative studies on the local isolates including their antigenic and genetic characterization using modern techniques

The part of the studies included into the present report covers the work which was done only on domestic birds chickens (mostly), turkeys and ducks The materials taken from the birds for virus isolation were mostly cloacal and tracheal swabs and sometimes some organs (lungs, trachea, bone marrow and lien) The studies were performed within the period June - December 1994 The geographic distribution covered the Nairobi district (including Kalolenı, Kabete and Westlands), Kariokor, Kerugoya, Kawangware Kericho, Kiambu, Nakuru, Embu, Thica, Chuka and Ruaraka

The swabs and homogenized organs were treated by antibiotics and passaged through 10-11-days-old embryonated eggs In the case of embryo death, allantoic fluid was harvested and examined in hemagglutination (HA) reaction If the embryos were alive the inoculated eggs were incubated up to six days post inoculation and then the allantoic fluid also was harvested and examined in HA reaction. The positive fluids, designated as HA agents, especially those bacterially contaminated, were sterilized by filtration through the Millipore membranes (45 um pores), passaged once again and examined in HA reaction (proof of the viral nature of the HA agents)

As a result of this surveillance, 121 hemagglutinating agents were isolated and passaged (Fig 3)

2 7 2 Serological identification of the hemagglutinating agents Isolated in Kenya in 1994 from domestic birds

The studies carried out in Kenya during the reported year include identification of more than 120 hemagglutinating (HA) agents which were isolated by the CI, Prof Philip N Nyaga and his team from domestic birds within the Central districts of Kenya in 1994

The identification of the above HA agents was carried during the visit of the PI to Kenya in March-May 1995, using reference antisera against all the APMV serotypes

The results have shown that all the isolates turned out to be NDV The main task was to investigate specific differences and peculiarities of the local Kenyan NDV isolates as compared to the Israeli isolates and the NDV reference strains For this aim, the preparation of chicken antisera against some of the local isolates was initiated which is in progress

2 7 3 Field surveillance performed during the visit of the PI to Kenya in 1995 and isolation of hemagglutinating agents

During the visit of the PI to Kenya, field surveillance was carried out in two places in safari Lake Nakuru and safari Lake Bogoria The main objective was attempts to isolate APMVs from feral birds flamingoes (Phoenicopterus ruber), in particular According to ecological data, each season flamingoes can choose different lakes In accordance with this, in lake Nakuru there were very few flamingoes while in the lake Bogoria there was massive residence (although the previous year the picture was opposite) The materials included faecal droppings, feathers and lake water samples were transported to the Department of Pathology and Microbiology in the Kabete campus and stored in -20 C

The materials were propagated in chick embryonated eggs and after passaging five HA agents were established According to preliminary identification with the reference APMV antisera which were brought to Kenya by the PI, the isolates appeared to be NDV All the isolates from the flamingoes as well as some isolates of 1994 from domestic birds have been transferred to Israel by the CI in fall 1995 for final identification.

3 Analysis of APMV-1 and APMV-2 viruses by means monoclonal antibodies against viral envelope proteins

3 1 Preparation of monoclonal antibodies against viral envelope proteins of NDV and Yucaipa viruses

The basic hybridoma technology (Kohler & Milstein, 1975) has been used, according to the specific schedule directed to paramyxoviruses (Orvell & Grandien, 1982) Preparation of the panel of monoclonal antibodies against each virus included the following stages

3.1.1. Preparation of purified virus material and its fractionation into envelope and internal viral proteins

(a) Preparation of the purified virus including the following stages clarification of allantoic fluid, pelleting of the virus, sucrose gradient centrifugation of the resuspended virus, final pelleting and resuspension.

(b) Crude fractionation of the virus proteins into envelope and internal protein fractions carried out by treatment of purified virions with the non-ionic detergent octyl-a-D-glucopyranoside (octyl-glucoside)

3 1 2 Immunization of BALB/mice with the viral envelope proteins

BALB/c mice were injected, first, intramuscularly and, then (2-% months later), intraperitoneally by the same envelope proteins fraction. The animals were sacrificed % days after the first booster and spleen cells were prepared

3 1 3 Production of hybridoma cell lines

The spleen cells were mixed with the BALB/c myeloma cell line in a serum-free medium. After washing and appropriate incubation the cells were distributed to individual wells of microplates and after additional incubation the clones were screened for antibody activity by ELISA technique Antibody-producing clones after further passaging in the microplate wells and, then, in tissue culture flasks, were injected into syngenic BALB/c mice, and ascitic fluid was obtained from individual mice 2-5 weeks later

3 1 4 Determination of antibody activities of the clones obtained by ELISA assay

All the clones obtained were titrated by ELISA, hemagglutination inhibition and neuraminidase inhibition tests and according to their activities were distributed into three groups strong, moderate and weak monoclonal antibodies

3 1 5 Determination of the MAB isotypes

This study was carried out using mouse monoclonal typing kit (The Binding Site Co) and according to the protocol supplied by the company The studies including 23 MABs have shown that all of them belong to the IgG, class, 17 MABs having isotype G1, and 6 MABs having isotype G2a

The prevalence of the iso-type G1, is in agreement with the published data (Orvell & Grandien, 1982)

3 2 Characterization of monoclonal antibodies (MAB) against NDV Establishment of the MAB panels

The MABs were prepared against Australia-Victoria strain of NDV by fusing spleen cells from NDV-immunized BALB/c mice with the mouse myeloma cell line SP2/0 (see the previous Annual Report) The viral envelope fraction obtained by octyl-glycoside treatment of purified virus preparation and containing hemagglutinin-neuraminidase (HN), fusion (F), and matrix (M) viral proteins was used for the immunization. The MAB-producing hybridomas were screened and isolated by ELISA test of cell culture medium samples and the MABs were produced in ascitic fluids The MABs were characterized by (a) radioimmune precipitation assay using double labeling of viral proteins and glycoproteins with amino acid and carbohydrate precursors, (b) antigen-capture ELISA test and (c) Western blot analysis Three MAB panels against HN, F and M proteins have been established which included 10, 7 and 15 MABs, respectively (Fig 4a, 4b 4c, 4d) These MABs precipitated single viral polypeptides were positive in antigen-capture ELISA test (Tab 16) but were negative in the Western blot, the latter suggesting that the viral antigenic epitopes were denatured by SDS during polyacrylamide gel electrophoresis

These MAB panels will be used for antigenic characterization of strains isolated in Israel during the last decade from different avian hosts as well as for revealing the hypothesized epitopes common to other APMVs this being one of the main aspects underlying the working hypothesis

The further characterization of the MAB panels was carried out by two directions determination of the MAB isotypes and determination of the non-overlapping groups of MABs within each of the three panels anti-matrix, anti-hemagglutinin-neuraminidase and anti-fusion MABs

3 2 1 The MAB panel against the matrix (M) protein determination of the non-overlapping groups

A panel of 18 MABS against the matrix (M) protein of NDV was obtained and their specificity towards the M protein was proven by radioummune precipitation assay, Western blot (in non-denaturating conditions) and antigen capture ELISA test Further studies were directed to antigenic epitope mapping of the M protein by means of this panel The epitope characterization was performed by competitive antibody-binding assay (Orvell & Grandien, 1982) by means of labeling of each MAB with biotin-amidocaproate-N-hydroxysuccinimide ester (Guesdon et al , 1979 At least, 5 clear groups non-overlapping or partially overlap ping have been determined, each including 4, 1, 1 8 and 1 MABs respectively (Fig 5) affiliation of the 3 remaining MABs was not clear Most of the MABs showed asymmetric competitive binding which is not due to the differences in antibody avidity, this phenomenon could be explained by structural peculiarities of certain epitopes of a respective group, this causing asymmetric steric hindrance for binding of the compared overlapping MABs All the above MABs turned out to be induced by structural epitopes formed in conditions of tertiary structure of the native M antigen.

3 2 2 The MAB panel against the fusion (F) protein determination of the non-overlapping groups

Nine from ten MABs of the panel have precipitated the F protein (Fig 6) This was proven by combination of the MAB binding to the lysate of the NDV-infected cells labeled by either amino acid or carbohydrate precursors One MAB did not precipitate any of the viral proteins from the lysate and its specificity was determined by means of the antigen-capture ELISA and competitive binding assay The Western blotting examination showed no binding of all the MABs with any of the viral proteins, this proving the structural nature of the epitopes which were inactivated as a result of their denaturation

Before performance of the competitive binding assay with biotin- labeled MABs for the F protein mapping, the saturated concentrations of the MABs were determined by the modified ELISA (Nishikawa et al , 1983)

The competitive binding assay (Fig 7) revealed two non-overlapping regions The first region included two non-overlapping sites (site 1 and site 2) and one site (site 1 2) overlapping with the site 1, on one hand, and the site 2, on the other hand. The second region included only one site (site 3) Consequently, the MAB F1a specific to the site 1 competed only with the MAB F1 2a specific to the site 1 2 The MABs F2a, F2b, F2c and F2d specific to the site 2 competed with each other and, besides, with the MAB F1 2a. The MABs F3a, F3b, F3c and F3d specific to the site 3 competed only with each other

All the defined F protein antigenic sites were highly sensitive to the denaturing agents, that fully considing with the literature (Russell et al , 1990)

Thus, the prepared MAB panel against NDV F protein made it possible to delineate three non-overlapping antigenic sites that is compatible with the published data. Namely, amongst the published results concerning characterization of MABs against NDV F protein (Russell & Alexander, 1983, Meulmans et al 1987 Abenes et al , 1986, Umino et al , 1990) there are two reports describing rather large MAB panels permitting antigenic mapping of the F protein (Abenes et al , 1986, Umino et al 1990) The former studies describe 3 antigenic sites revealed by the competitive binding test but 4 sites revealed by the selection of MAB-resistant mutants (one of the above three sites was split into two non-overlapping sites) The latter paper like ours describes 3 antigenic sites There-fore, it seems that the presence of three non-overlapping antigenic sites is characteristic for the NDV

The F protein of paramyxoviruses is accounted as antigenically stable (Abenes et al (1986, Russell et al , 1990) In particular, the analysis of the published data on NDV concerning the relation between the stable and variable epitopes has given the following account in the case of the F protein 504 of the epitopes were stable as compared to the 384 in the case of the HN protein (Russell et al , 1990) However, the presented results are rather in a certain contra-diction with such conclusion namely, contrasting differences in the stability were observed when both the sites and the epitopes within the same site were compared In general, the sites 1 and 3 can be considered more stable than the sites 1 2 and 2 The comparison of the obtained data with those by Abenes et al (1986) shows that the number of the stable epitopes in the latter publication was significantly higher (four from six) than in our studies (two from nine) The reason for such discrepancy is probably due to the fact that our collection of the NDV strains used in the studies was considerably higher (63 against 17)

The data having demonstrated a significantly higher variability of the NDV F protein, as opposed to the accepted views about its rather high stability should be taken into account in anti-NDV vaccination policy

3 2 3 The MAB panel against the against hemagglutinin-neuraminidase (HN) protein determination of the non-overlapping groups

The panel of 14 MABs against HN protein whose specificity to the HN was determined by ELISA was examined by radioimmunoprecipitation assay using double labeling with [^{35}S]-methionine and [^{3}H]-glucosamine of the virus growing in the NDV-infected BHK cells All the MABs gave positive precipitation with both the labels (Fig 8) At the same time, all the MABs gave negative reaction in the immunoblotting test if the test was performed in reducing conditions, this proving the structural nature of the epitopes which were inactivated as a result of their denaturation. By performing the immunoblotting test in non-reducing conditions, it was found that 13 MABs from the panel reacted with the protein having electrophoretic mobility corresponding to the MW 51-56K while 3 of them reacted also with the protein having electrophoretic mobility corresponding to the MW 69-72K and only one MAB reacted with the protein of MW 69-72 only Such phenomenon was described by Samson et al (1986) who provided the explanation that in reducing conditions the HN forms two conformational variants - normal (MW 69-72K) and more compact one having a higher electrophoretic mobility (corresponding to the MW 51-56K)

Like in the previously described with the anti-F MABs, before performance of the competitive binding assay with biotin-labeled anti-HN MABs, the saturated concentrations of the MABs were determined by the modified ELISA (Nishikawa et al , 1983)

The competitive binding assay (Fig 9) revealed 6 main groups The group 1 includes two MABs - HN1a and HN1b - highly competing with each other and with the adjacent group 1 2 and not competing with all the other MABs The group 2 includes 2 MABs which totally inhibit each other and all the MABs of the group 1 2 Thus, the above MABs reveal upon the HN protein three closely located antigenic sites from which the sites 1 and 2 are non-overlapping while the MABs to the epitopes of the site 1 2 react with both the epitopes of the site 1 and the site 2 The rest of the MAB panel - 5 MABs - are distributed within 3 groups designated as group 3 (1 MAB), group 4 (3 MABs) and group 5 (1 MAB) The groups 3 and 5 are not cross reacted at all As to the MABs of the group 4 two of them - HN4b and HN4c - compete with each other and compete with the MAB HN4a by asymmetric way, e g they inhibit to the binding of the MAB HN4a with the antigen while the MAB HN4a has no influence on the ability of the labeled MABs HN4b and HN4c to bind to the antigen.

The MABs specific to the sites 1 and 1 2 inhibit the viral hemagglutinating (HA) activity without influence on the neuraminidase (Nase) activity Contrary to it, the MABs specific to the site 2 inhibit the Nase activity without influence on the HA activity The MAB specific to the site 3 - HN3a - inhibit both the HA and Nase activities

Thus, the prepared panel of MABs against the HN protein reveals upon the HN glycoprotein 5 non-overlapping antigenic sites and one site (HN1 2) overlapping with sites 1 and 2 This panel is the richest one from those described in literature

3 2 4 The MAB-mediated analysis of NDV strains isolated in Israel from domestic and feral birds analysis of contrasting variability amongst the strains

Twelve reference strains and 51 recently isolated Israeli strains of NDV have been studied by means of 3 panels of MABs against hemagglutinin-neuraminidase (HN), fusion (F) and matrix (M) viral proteins containing 10, 7 and 6 MABs, respectively The MAB-producing hybridomas were prepared against Australia-Victoria (AV) strain of NDV by fusing spleen cells from NDV-immunized BALB/c mice with the mouse myeloma cell line SP2/0 The viral envelope fraction was used for the mice immunization. The reference strains included velogenic, mesogenic and lentogenic ones isolated within 1933- 1970 period from domestic fowl The native NDV strains have been isolated recently (1984-1994) from various avian species including poultry (chickens and turkeys), feral (wild and show pigeons, starlings, gulls eagle, cattle egret), water-fowl (mallards and coots), and cage (parrot) birds The antigenic and/or functional relatedness between all the above NDV strains was examined by the pattern of their binding to each MAB belonging to either panel The binding was measured by ELISA technique

The results revealed two contrasting indications amongst different strains a wide spectrum of variability against stable conservativity in the MAB binding patterns (Fig 4) Some of the "stable" strains did not differ from the homologous AV NDV although the gap between the dates of their isolations reached more than 50 years There was no correlation between the MAB binding pattern and the strain pathogenicity, host specificity, geographic location and chronology of isolation. Together with this there was no strong correlation between the data related to different panels for example some strains could be grouped according to the binding pattern with MABs belonging to the HN panel but this grouping was not compatible with the results related to the other panel(s) On the background of the diverse variations, some of the NDV epitopes turned out to be highly conserved

DISCUSSION The described combination of the MAB panels against all 3 viral envelope proteins and 6 3reference and native strains of NDV is a solid basis for the analysis of NDV antigenic epitope pattern and variability All the MABs, except 3 anti-M ones, were proven to be distinct According to the Western blot test, all the MABs appeared to be primed by conformational epitopes Similar results have been described (Samson et al , 1988 Yusoff et al 1988) Among all the panels, anti-M MABs showed a

higher stability and anti-F MABs showed a higher variability as compared to anti-HN MABs The latter is rather in contrast to the results obtained in the other labs (Abenes et al , 1986, Meulemans et al , 1987, Russell et al , 1990) On the background of the diverse variations, some of the NDV epitopes turned out to be highly conserved

The absence of correlation between the MAB binding pattern and the strain pathogenicity, host specificity, geographic location and chronology of isolation is in accordance with the literature (Iorio et al. 1986, Russell et al , 1990) However, such contrasting combination of high variability with stable conservativity among the NDV strains is a unique point of the present studies Namely, some of the "stable" strains did not differ from the homologous AV NDV although the time gap between their isolations is more than 50 years

4 Sequencing of APMV genes

This became to be the main effort that started from the organization of the experimental set-up for the sequencing studies

4 1 The strategy

The strategy of the research was based on the direct sequencing of APMV virion RNAs by "gene walk" tactics using synthesis of complementary DNA on the virion RNA matrix by means of reverse transcriptase reaction and the sequence determination by the dideoxynucleotides chain termination method by (Sanger et al 1977) The principal difficulty of the chosen strategy was that NO part of the genome of avian paramyxoviruses except NDV has been sequenced so that initial information needed for the synthesis of the first primer in order to start "gene walk" was absent Therefore we have employed the fact that all the genes of NDV were sequenced comprehensively (Chambers et al 1986a, 1986b, Ishida et al 1986, Jorgenson et al , 1987, McGinnes & Morrison, 1987, Wemers et al , 1987, Yusoff et al , 1987 McGinnes et al , 1988, Gotoh et al , 1988, Schaper et al , 1988, Millar et al , 1988) Namely, the data of 1% different strains have been studied (Sakaguchi et al , 1989, Toyoda et al , 1989) The alignment of the respective sequences showed a number of genomic portions in which there was no sequence differences between the compared viruses and which, hence, could be considered as "conserved" parts Based on the assumption that these "conserved parts" could be common not only to different strains of NDV but to some other serotype(s) of APMV, in general, we have ordered the first primers complementary to the genomic viral RNA (i e in mRNA-sense) in some of such "conserved" parts belonging mostly to the HN and the F genes Thus, we hoped to use these primers for "fishing" the assumed conserved portions in certain other APMV(s) The experiments, indeed, have shown that in several cases the annealing of certain virion RNAs with heterologous primers isolated from various APMVs did occur followed by the subsequent synthesis of cDNA complementary to the virion RNA This resulted in determination of some sequences in the case of several APMVs Some of the fluorographic pictures of the sequences were difficult to read because of either very faint bands, or interference with additional sequence(s) Therefore, it needed a long exposure of fluorographic films and more than one variant of reading By overcoming these obstacles, the sequences of certain portions of APMVs have been obtained so that the next steps could be performed by real "gene walk" manner using homologous primers The corresponding consequtively sequenced portions were aligned using NUCALN software program which resulted in getting longer sequences Then, after 2- 3steps of the "gene walk" the found sequences could be transferred via E-mail to the European Molecular Biology Organization (EMBO) DataBank in Heidelberg (through the Institute of Virology, Marburg University, Germany) for comparison with all the viral sequences stored in the DataBank, the program FASTN being used The viral sequences of the EMBO library (EMBL) which showed the best identity scores were aligned with the analyzed pieces and the highest percentage of homology was the basis of the APMV gene identification. The next step was determination of the open reading frame (ORF) which, first, supported a proper gene portions' identification putative determination of trascriptional and translational parts (initiation and termination codons), intergenic regions and con census sequences, and, secondly, provided deduction of the amino acid sequences from the nucleotide ones with possible further detection of potential glycosylation sites (for hemagglutinin-neuraminidase and fusion glycopro-

teins) and some other special sites (cleavage etc)

The principal difficulty of the chosen strategy was that no part of the genome of avian paramyxoviruses, except NDV, has been sequenced, so that initial information needed for the synthesis of the first primer in order to start "gene walk" was absent. Therefore, we have employed the fact that all the genes of NDV were sequenced comprehensively (Chambers et al , 1986a, 1986b, Ishida et al, 1986 Jorgenson et al , 1987 McGinnes & Morrison, 1987, Wemers et al , 1987, Yusoff et al , 1987 McGinnes et al , 1988 Gotoh et al 1988 Schaper et al , 1988, Millar et al 1988) Namely, the data of 1% different strains have been studied (SakaguchI et al , 1989 Toyoda et al , 1989) The alignment of the respective sequences showed a number of genomic portions in which there was no differences and which, hence, could be considered as con-served parts Based on the assumption that these "con-served parts" could be common not only to different strains of NDV but to some other serotype(s) of APMV, in general, we have ordered the first primers complementary to the genomic viral RNA (i e in mRNA-sense) in four such "conserved parts" belonging to HN gene and in eight parts belonging to the F gene Thus, we hoped to use these primers for "fishing" the assumed conserved portions in certain APMV(s)

The experiments, indeed, have shown that in several cases the annealing of certain primers with heterologous virion RNAs isolated from various APMVs did occur followed by the subsequent synthesis of cDNA complementary to the virion RNA This resulted in determination of some sequences in the case of 1831, Pk/Neth, Sw/Naz, Goose/Del and Dove/Tn viruses Some of the fluorographic pictures of the sequences were difficult to read because of either very faint band, or interference with additional sequence(s) This was a result of annealing of heterologous primers and RNAs Therefore it needed a long exposure of fluorographic films and more than one variant of reading

4 2 The methodical approach

It has been decided to carry out this part of the studies using the "gene walk" strategy of the direct sequencing of virion RNAs, using synthesis DNA on the virion RNA matrix by means of enzyme reverse transcriptase and the sequence determination by the di-deoxynucleotides chain termination method by (Sanger et al 1'77)

The studies included the following steps

A Inoculation of chick-embryonated eggs by virus
B Harvesting of the allantoic virus and its titration.
C Purification of the virus
a) clarification of allantoic fluid,
b) pelleting of the virus from the allantoic fluid by ultracentrifugation
c) sucrose gradient ultracentrifugation of the resuspended pelleted virus, collection of the virus- containing band, its homogenization and ultrasonic treatment,
d) final pelleting of the virus by ultracentrifugation,
e) ultrasonic treatment of the purified virus preparation
D Isolation of viral RNA using proteinase K and phenol-chloroform treatment of the purified virus preparation.
E Purification of synthesized olygonucleotides (primers) by means of Sephadex columns
F Sanger's reaction
a) annealing reaction between virion RNA and primer;
b) synthesis of DNA upon the virion RNA matrix annealed with appropriate primer using reverse transcriptase, normal and di-deoxynucleotides and ^{35}S-labelled ATP,
G High voltage electrophoresis of the products of the sequencing reaction and processing of the gels
a) fixation and drying,
b) fluorographic exposition,
c) development of the exposed films
H Reading of the sequence data
I Computation of the sequence data.

4 3 Sequencing of particular APMVs' genes

4 3 1 Enders-Ruckle virus (ERV)

The main problem of the direct sequencing of an unknown virus - the choice of the initial primer was solved by using the initial information about identification of ERV as avian parainfluenza of serotype 2 (PIV-2) Therefore, the recently published data on sequencing of hemagglutinin-neuraminidase (HN) gene (Precious et al , 1990) and nucleoprotein (NP) gene (Koevamees et al , 1989) of PIV- 2 virus were employed The choice of the respective primers was done on the basis of those portions of the corresponding genes which showed maximal homology when the sequences of 3 closely related viruses PIV- 2, SV5 and mumps viruses (Precious et al , 1990, Elango et al 1989) have been compared, i e the portions of the above genes with identical sequences for all the three viruses were chosen for the synthesis of primers The sequencing reactions were successful, and several "gene walk" steps using then homologous primers were per-formed The corresponding sequenced portions were aligned using NUCALN software program which resulted in getting a resulting pieces of 1633 bases in the case of the primer to the HN gene (HN-L portion) and 615 bases, in the case of the primer to the NP gene (NP-P portion) These pieces were sent via E-mail to the European Molecular Biology Organization (EMBO) DataBank in Heidelberg (through the Institute of Virology, Marburg University, Germany) for comparison with all the viral sequences stored in the DataBank, the program FASTN being used. The best identity scores with high percentage of homology with ERV appeared to be in the case of different strains (SBL, Enders and Miyahara) of mumps virus the differences between ERV and mumps viruses were within usual strain-specific differences (Fig 10 & 11) Together with this, according to the alignments between the compared ERV and mumps virus sequences, it was possible to determine the localization of the sequenced pieces within the viral genome Namely, it appeared to be that one piece covered about three quarters of the 3' part of the HN gene and 5' part of the adjacent L gene (Fig 12) Another portion covered the 3' part of the NP gene (Fig 13) and the 5' part of the adjacent P gene (Fig 14) [all the gene portions' designations are done in the "mRNA sense"] In this case, the sequence portions had to contain non-translational down-stream portions of the HN and NP genes, respectively, and non-translational up-stream portions of the P and L genes, respectively The above conclusion could be clearly demonstrated by the results on the determination of open reading frames (ORF) and amino acid sequences in both the portions Namely, the terminating codon, down-stream transcription part, TATA-box, and poly-A tail of the end of HN and NP genes respectively on one hand, and, putative intergenic region and consensus sequences of the beginning of the L and P genes, respectively, on the other hand, can be determined This work is now in progress

Thus the partial direct sequencing of ERV vRNA showed high percentage of homology with different strains of mumps virus in certain portions of NP (85-92%) P (79-84%) HN (92-97%) and L (92-95%) genes

On the basis of these data, together with the results on antigenic characterization, the ERV was identified as avian mumps-like virus (AmuV), that having been accepted by the International Committee on Virus Taxonomy

4.3.2. APMV serotype 1, APMV-1/racing pigeon/Israel/84

4 3 2 1 HN gene

Annealing of virion RNA of this virus with a primer synthesized on the basis of a "conserved" portion of the HN gene of NDV gave clear result. The "gene walk" was performed and, after 4 steps a sequence of a portion of 75 3bases was determined which (Fig 15) Using the above described HUSAR Program, this portion was shown to have a high per cent of homology (84%) with the Newcastle disease virus (Fig 16)

4 3 2 2 L gene

Annealing of virion RNA of this virus with a primer synthesized on the basis of a "conserved" portion of the F gene of NDV gave clear result. The "gene walk" was performed and, after 4 steps, a sequence of a portion of 948 bases was determined (Fig 17) Quite unexpectedly, while using the HUSAR Program described previously (see the previous Annual Reports), this portion was shown to have a high per cent of homology (894) with the L gene (but not the F gene!) of the NDV (Fig 18) The reason for that may be due to the presence on the L gene of the above APMV-1/racing pigeon/Israel/84 virus of the sequence which has a higher degree of complementarity to the selected primer as com- pared to the conserved part of the F gene on the basis of which the primer was selected

4.3 3. APMV serotype 3a, APMV-3a/turkey/Wisconsin/65

4 3 3 1 P gene

Annealing of virion RNA of this virus with a primer synthesized on the basis of a "conserved" portion of the F gene of NDV gave a clear result and after three steps of "gene walk" a gene portion of 826 bases was obtained (Fig 19) By means of the HUSAR Program, it was possible to determine that this sequence belongs to the P gene, but the alignment of this sequence with the P gene of any PMV was not obtained However, using the Nucaln Program, this sequence was compared with the sequence related to the APMV-3b/parakeet/Netherlands/441 which was obtained by means of the same initial primer and identified as belonging to the P gene This comparison showed good alignment between both the sequences, which supported the conclusion that the found sequence concerns the P gene (Fig 20)

4 3 3 2 F gene

Annealing of virion RNA of this virus with a primer synthesized on the basis of a "conserved" portion of the F gene was successful After three steps of the "gene walk", a sequence of 962 bases was determined which, by using the above-described schedule, was identified putatively as a portion covering the 3' part of the F gene and the start of the adjacent down stream gene which could be potentially either HN or SH (small hydrophobic) gene (Fig 21) The ORF determination is in progress

4 3 3 3 L gene - the END of the genome

Annealing of virion RNA of this virus with a primer synthesized on the basis of a "conserved" portion of the HN gene of NDV gave a clear result and after three steps of "gene walk" a gene portion was obtained which turned out to belong to the end of the whole genome (Fig 22, Fig 23) The comparative analysis (alignment) of the portion could not be performed because of the small size of the portion

4 3 4 APMV serotype 3b, the prototype virus APMV-3b/parakeet/Netherlands

Annealing of virion RNA of this virus with a primer synthesized on the basis of a "conserved" portion of the HN gene was very poor After a long exposition it was still very difficult to read unequivocally the obtained sequencing fluorograph and three varying primers were synthesized according to different reading versions Finally, the "gene walk" was performed and, after two steps, a sequence of 687 bases was determined which, by using the above-described schedule, was shown to have the highest homology with the P (phosphoprotein) gene of human mumps virus Accordingly, by aligning with reference gene the determined sequence was identified putatively as a portion covering the 3'-part of the P gene and the start of the adjacent M (matrix) gene The ORF has been determined, and terminating codon, TAAG-box, poly-A tail, intergenic region and consensus sequence were putatively detected (Fig 24)

4.3.5. The PMV-3/swine/Nazareth/80 antigenically similar to the APMV-3b/parakeet/Netherlands/75

The same primers as those used in the case of the APMV-3b/parakeet/Netherlands/75 were used successfully and the obtained sequence of 669 bases was shown to have the highest homology with mumps virus P gene (Fig 25) Both the viruses avian APMV-3b/parakeet/Netherlands/75 and the mammalian PMV-3/swine/Nazareth/80 turned out to be very closely related to each other (Fig 26)

4 3 6 APMV serotype 6, APMV-6/duck/Hong Kong/75, L gene - the END of the genome

Annealing of virion RNA of this virus with a primer synthesized on the basis of a "conserved" portion of the F gene of NDV gave a clear result and, after two steps of "gene walk" a gene portion was obtained which also turned out to belong to the end of the whole genome (Fig 27, Fig 28) The comparative analysis (alignment) of the portion could not be performed because of the small size of the portion.

4 3 7 APMV serotype 7 prototype virus APMV-7/dove/Tennessee/75 M gene

4 3 7 1 M gene

Annealing of virion RNA of this virus with a primer synthesized on the basis of a "conserved" portion of the NDV HN gene was rather poor Finally after three steps of the "gene walk", a sequence of 971 bases was determined which, by means of the above-described schedule, was shown to have the highest homology with the M (matrix) gene of human parainfluenza virus, serotype 2 (PIV-2) [Fig 29] Accordingly the determined sequence was identified putatively as a portion covering the 3'-part of the M gene and the start of the adjacent F (fusion) gene The ORF was determined and the terminating codon TATA-box poly-A tail, intergenic region and con-census sequence were putatively detected (Fig 30)

4 3 7 2 F gene

The sequence which after the usage of the initial primer was determined as belonging to the M gene (see Annual Report No 3) was further studied using the "gene walk" strategy Since the previous piece was shown to have the highest homology with the M (matrix) gene of human parainfluenza virus, serotype 2 (PIV-2), the start of the adjacent F (fusion) gene was planned to be determined After 5 steps of the 'gene walk' a piece of 1124 bases was determined (Fig 31) which makes about 2/3 of the F gene

4 3 7 3 HN gene

Annealing of virion RNA of this virus with a primer synthesized on the basis of a "conserved" portion of the NDV F gene gave clear result. Finally, after four steps of the "gene walk" a sequence of 1215 bases was determined (Fig 32) which, by means of the above- described schedule was shown (paradoxically!) to have the highest homology with the HN (hemagglutinin-neuraminidase) gene of NDV (Fig 33) Alignment showed that the sequenced portion belong to the middle of the HN gene The continuation of the "gene walk" experiments is in progress

4 3 8 APMV serotype 8, APMV-8/goose/Delaware/76

Annealing of virion RNA of this virus with two primers synthesized on the basis of "conserved" portions of the HN and F genes was poor After a long exposition, it became possible to read the obtained sequencing fluorographs One of the pieces consisted of 259 bases, another was of 149 bases (Fig 34 and 35) Both the pieces turned out to be not sufficient for determination of the homology by the HUSAR DataBank.

4 3 9 APMV serotype 9, APMV-9/duck/New York/80, HN gene

Annealing of virion RNA of this virus with a primer synthesized on the basis of a "conserved" portion of the F gene was poor However, after a long exposition, it became possible to read the obtained sequencing fluorograph and to proceed the "gene walk" strategy After two steps, a sequence of a portion of 779 bases was determined (Fig 36) which, by using the above-described schedule, was shown to have the highest homology with the L (polymerase) gene of NDV (Fig 37) The comparative analysis of both the viruses is under studies

4 3 10 APMV undetermined serotype, APMV-?/pigeon/Otaru/76, L gene

On the basis of a "conserved" portion of the NDV F gene and the corresponding primer, the annealing of virion RNA of this virus with a primer synthesized on the basis of a "conserved" portion of the F gene of NDV gave a clear result and after five steps of "gene walk" a portion of 1361 bases was obtained (Fig 38) By means of the HUSAR Program, it was possible to determine that this sequence showed maximal homology with the L gene of the NDV virus (Fig 39)

5. Impact, Relevance and Technology Transfer

The scientific impact of the project is due to the fact that paramyxoviruses as the main representatives of the negative strand viruses with nonsegmented genome possess some unique peculiarities concerning structure and function of their genome and properties of their proteins, HN glycoprotein, in particular Together with this, these viruses are of high practical importance The PMVs include pathogens for human such as mumps and parainfluenza, apart from respiratory syncitial and measles viruses belonging to other genera of the family Paramyxoviridae (there are views about association of measles-like viruses with some latent infections having causal connection with multiple sclerosis)

In accordance with this the studies on the basic knowledge about HN of the recently isolated APMV serotypes have been an important part of the project impact. The genetic part of the project, namely, data on the nucleotide sequencing of certain genes of the prototype strains of different APMV serotypes is the first results of such work which contributed to the establishment of the APMV taxonomy and to better understanding of phylogenetic relationships amongst different APMVs and between the APMVs and the other paramyxoviruses These data would provide also some practical application of the results in the field of the vaccine and diagnostics development, namely, in preparation of polyvaccine against APMVs and probes for group-specific and serotype-specific diagnostics

The usefulness of the findings for the collaborating country is due to the fact that the APMVs apart from NDV whose economic impact is very high, have been isolated quite recently and, hence, the knowledge about them is scanty, while they may be a potential threat for poultry However, they present a very lucky experimental model for studying antigenic and functional properties of the HN glycoprotein - a key-factor of the PMV antigenicity and pathogenicity The virological surveillance performed in Kenya, which is the first one in the country made evident impression upon the Nairobi University authorities, which facilitated performance of the scientific activities of the CI The collaboration, hence, has strengthened the Department of Veterinary Pathology and Microbiology The budget allocated to the Department permitted to purchase the necessary equipment. The personnell (M. Macharia, BSc, and J Kassiiti, BSc, have acquired the necessary experience in the course of the performance of the project The present project is the first CDR-supported project in the University of Nairobi, that strengthening the CI's Department and the Faculty

During the last two years of the project's performance, some new developments appeared to be that was depending on the simultāneously performed work on the collaborative studies with Kazakhstan carried out in the limits of the project also supported by A I D The results obtained in the limits of both the projects were in agreement that permitted to make some general conclusions, especially in the field of viral ecology concerning the global (intercontinental) spread of paramyxoviruses

6. Project Activities and Outputs

Mutual travels of PI and CI were realized in the following way The travel of the PI to the University of Nairobi was realized in April-May 1995 During this visit, new techniques for the APMV diagnostics and identification were established in the collaborating labs, and all the HA agents isolated earlier were identified Besides some field expeditions for the collection of swabs and other materials from feral birds were undertaken. Then, there were two visits of the CI to Israel – in 1995 and 1996 During those visits, CI was busy in the studies on newly isolated viruses using all the modern techniques used in the PI's laboratory

Participation in the scientific meetings

The results of the were reported on the following meetings

1 The IXth International Congress of Virology, Glasgow Aug 8-13, 1993
2 The 3d Congress of the European Society for Veterinary Virology, Immunology and Viral infections, Interlaken, September 4-7, 1994
3 The Annual Meeting of the Israel Society for Microbiology, Rehovot, February 14-15, 1994
4 The 1st Conference of the Israel Society for Biochemistry and Molecular Biology, Ramat-Gan, Israel,
5 March 21-22, 1994
6 The Annual Meeting of the Israel Society for Microbiology, Haifa, February 6-7, 1995
7 The local Meeting at Nairobi Univercity May, 1995
8 The Annual Meeting of the Israel Society of Microbiology Jerusalem February 5-7 1996
9 The VIIIth International Symposium of Veterinary Laboratory Diagnosticians, Jerusalem August 4-9, 1996
10 The Xth International Congress of Virology, Jerusalem, Israel, August 11-6, 1996
11 The Conference on Infectious Diseases, Tanzania, Dar-a-Salam, 1997
12 The Annual Meeting of Israel Society for Biochemistry and Molecular Biology Tel Aviv January 20-23, 1997
13 The Annual Meeting of the Israel Society of Microbiology Tel Aviv April, 1997
14 The Annual Meeting of the Israel Society of Microbiology, Tel Aviv February 9-10 1998
15 The 1st International Symposium on Turkey disease, Berlin-Steglitz February 19-21 1998

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In reviewed periodicals and books

1 Shihmanter, E , Alexander, D , Weisman, Y , Collins, M. & Lipkind, M (1995)
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2 Panshin, A., Shihmanter, E , Orvell, C , Weisman, Y , & Lipkind, M. (1995)
Characterization of Newcastle disease virus field strains by means of 3 panels of monoclonal antibodies analysis of variation regularities
In ***Immunobiology of Viral Infections*** (Editors M Schwyzer, M. Ackerman, G Bertoni, R. Kocherhans, K. McCullough, M Engels, R. Wittek & R. Zanoni), pp 475-478

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Comparative Immunology, Microbiology and Infectious Diseases, 20 177-189

8 Panshin, A., E Shihmanter, Y Weisman, C , Örvell & M. Lipkind. (1998)
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1 Lipkind M., H -D Klenk, C Orvell, E Shihmanter, A. Panshin, R. Rydbeck, Y Weisman & R. Rott (1993)
Antigenic and genetic identification of Enders-Ruckle virus an avian paramyxovirus
Proceedings of the IXth International Congress of Virology, Glasgow, Scotland, pp 117

2 Shihmanter E , M. Lipkind & Y Weisman (1994)
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3 Panshin A , E Shihmanter, C Orvell, M. Lipkind & Y Weisman (1994)
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Proceedings of the VIIIth International Symposium of Veterinary Laboratory Diagnosticians, Jerusalem, Israel, pp 42

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14 Panshin A & Kostyukov M (1996)
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16 Shihmanter E , Panshin A., Örvell C , Weisman Y and Lipkind M. (1997)
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7. Project Productivity

Mainly, all the proposed goals have been accomplished, although there was a certain shift in priorities This was due to the above-mentioned fact that the proposal was submitted in 1987 and, by some circumstances, the date of August, 1991, was determined by A. I D as the starting point of running the project. During this period (between the submission of the proposal and the start of the project), new developments in the field, including those published in the world and some data obtained in PI's lab, had to be taken into account. In fact, all the objectives, which were in the original proposal, have remained but some new topics were included and new trends were considered. This was expressed in certain changes in the research strategy (redistribution of priorities for certain objectives) Namely, much more emphasis was done to the studies on avian paramyxoviruses on the genetic level using sequencing technology Besides, new viruses, which were of especial interest to some tasks of the research, were included into the studies Therefore, certain modifications included into the work plan were realized in the execution of the project.

Requests for A. I. D or BOSTID Actions

First of all, many thanks for cooperation

The only suggestion for a better assistance of AID/SCI or BOSTID staff in promoting the project productivity concerns a more efficient reaction (at least in the form of simple acknowledgment) to the reports or any correspondence from the PI

The best example concerns the previous Annual Report, which was sent to AID/SCI in December 14 1992 An acknowledgment letter from A. I. D dated by January 4 1993 was delivered to the PI on October 18 1993 and the postmark upon the envelope in which the letter was enclosed showed "OCT 12'93" That means that the time reaction ("stimulus-response") period was about 10 months from which the way from Washington to Israel took 6 days The conclusion is that the rest of the time (10 months minus 6 days) the responding letter was traveling within the A. I. D area. Besides in this letter it was written "either a member of our staff or a member of the NAS/BOSTID staff will be in contact with you once the report is reviewed" Since then nobody has been in contact with PI.

8. Future Work

During the execution of the project, close and mutually beneficial connections have been established between the collaborating institutions, which provided current collaboration after official expiration of the project This is based on the continuous work on the comparative studies on the APMV isolations made in Israel, Kenya and Kazakhstan. The latter country is still under current support of the A.I.D – CAR program. The studies will be concentrated on the establishment of new techniques in the Collaborating institution. The results obtained are planned to be submitted to local and international meetings The comparative studies provide a unique opportunity to achieve some scientific conclusions of transcontinental transfer of viruses by migrating birds, that being of high ecological and epizootiological importance

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10. Appendices

FINAL REPORT

Covering period September 1991 - September 1998

"Antigenic and Functional Properties of Hemagglutinin-Neuraminidase Glycoprotein of Avian Paramyxoviruses"

Appendices

Publications

Immunobiology of viral infections Proc 3rd Congress Europ Soc Vet Virol (1995) 460-463 Edited by M Schwyzer M Ackermann G Bertoni, R Kocherhans K McCullough M Engels R Wittek and R Zanoni

ANTIGENIC AND FUNCTIONAL CHARACTERIZATION OF AVIAN PARAMYXOVIRUSES SEROTYPE 2 (APMV-2, "YUCAIPA-LIKE") ISOLATED FROM DOMESTIC AND WILD BIRDS IN ISRAEL ECOLOGICAL AND EVOLUTIONARY CONCLUSIONS

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ABSTRACT

Thirty-three viruses of APMV-2 serotype isolated in Israel from domestic and wild birds during epizootic of a respiratory disease in 1979-1981 were studied comprehensively in comparison with a set of reference APMV-2 viruses using (1) hemagglutination inhibition cross reaction test, (2) elution-hemagglutination pattern, and (3) pattern of migration of viral proteins in polyacrylamide gel electrophoresis The results demonstrated considerable heterogeneity amongst the local isolates by all the three criteria By the 1st criterion the isolates could be distributed into 4 main groups, according to the relatedness to different reference APMV-2 viruses By the 2nd criterion, the isolates differed strikingly from those showing very fast elution kinetics up to those showing the absence of elution By the 3d criterion, all the isolates could be distributed into two groups differing by the position of the F and M proteins and the band density of the HN protein However there was no correlation between the distributions of the isolates made in accordance with the above three criteria Besides, there was no significant correlation between the differences found and the chronology of isolation of the viruses The results indicated that there may have been simultaneous co-circulation of different APMV-2 strains in the local avian reservoir at the beginning of the epizootic rather than temporal accumulation of variants as a result of antigenic drift of an "ancestor' virus which caused the initial infection Analysis of ecological evidence permits to suggest that the initial APMV-2 virus was transferred into the local avian reservoir from 'abroad" probably via migrating birds and the "imported" virus was already heterogeneous Since the earliest Israeli APMV-2 isolation preceding the epizootic in poultry was that from wild ducks it is tempting to speculate about interspecies transfer of the APMV-2 virus from wild migrating to local domestic birds

INTRODUCTION

APMV-2 viruses (prototype APMV-2/chick/California//Yucaipa/56 [Bankowski et al , 1960]) have been shown to be widespread all over the world having been isolated both from domestic and wild birds (Alexander, 1982, 1986, Goodman & Hanson, 1988)
The first isolation of APMV-2 virus in Israel was done in 1979 from mallard ducks (Lipkind et al , 1982b) Soon after, many poultry farms in Israel were affected by a respiratory disease characterized by signs ranging from mild rales to severe respiratory distress including conjunctivitis, nasal discharge, sinusitis, pneumonia and drop in egg production Morbidity approached 100% while mortality varied from 5% to 90% Investigation of poultry farms and surveillance of feral birds has resulted in isolation of more than 70 APMV-2 viruses from all parts of Israel from turkeys, chickens, coots (*Fulica atra*) and cattle egret (*Ardeola ibis*) (Lipkind et al , 1981, 1982a, 1982c)
The topic of the present work was to compare properties of selected Israeli isolates with reference viruses

MATERIALS AND METHODS

Ten reference APMV-2 viruses and 33 native isolates were used in the studies The latter were isolated and identified as described (Lipkind et al , 1979, 1982a, 1982b, 1982c)
Hemagglutination inhibition (HI) test was done as described (Lipkind et al ,1979) Statistical

treatment of the HI test data was performed as described (Lipkind & Shihmanter, 1986, Lipkind et al , 1986)
Structural polypeptide analysis was done by polyacrylamide gel electrophoresis (PAGE) as described (Alexander and Collins, 1981)
Virus elution from red blood cell was estimated as described (Spalatin et al , 1970) According to the estimation, the viruses were placed into 4 groups as follows non-eluters (no elution after 24 hr), partial eluters (incomplete elution at 24 hr), moderate eluters (full elution at 24 hr) and fast eluters (full elution within 4 hr)

RESULTS

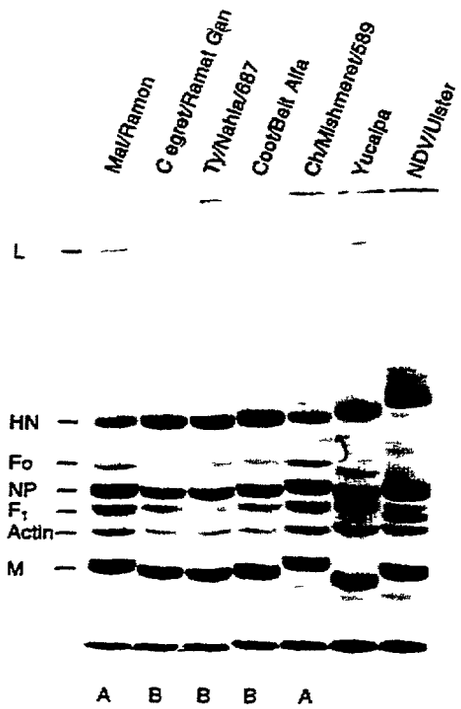
HI Tests Amongst the reference viruses, the cross reactivity varied considerably The Israeli isolates showed substantial heterogeneity if tested by antisera against some reference viruses, including Yucaipa virus This heterogeneity was in contrast to the results obtained with antisera against two selected Israeli viruses In some cases, the heterologous HI titer was higher than the homologous one As such a phenomenon was observed with all or most of the reference antisera reacted with a certain virus, this might reflect a particular sensitivity of the viral antigen (the phenomenon of "avidity")

Elution properties There were considerable variations in elution rate among both the reference strains and the isolates As to the reference strains, only Haneda and Bangor behaved as fast eluters, the others showed either no or only partial elution after 24 hr Thirty-three field isolates showed considerable heterogeneity Most of the strains (23) were partial or moderate eluters, five isolates were non- eluters and five classed as fast eluters

Structural polypeptides Purified preparations of the native isolates were subjected to SDS-PAGE in comparison with the Yucaipa and NDV/Ulster viruses (Fig 1) The overall polypeptide patterns of the isolates were similar (the same number and approximate size of the proteins but differed from the reference Yucaipa virus in migration rate of the major polypeptides However, more detailed examination revealed slight differences amongst the isolates which were expressed in the position of the F and the M proteins and the band density of the HN protein By these indications, all the studied viruses can be distributed into two groups The group A included viruses whose F glycoprotein migrated faster, the M protein migrated more slowly and the density of the HN protein band was significantly lower as compared to the respective values of the viruses belonging to group B (Fig 1)

DISCUSSION

The library of the Israeli APMV-2 isolates (more than 70 isolates) used in the present studies is a unique one since most of the isolates were obtained during the 3-years-long epizootic which spread through most of the poultry farms in the country This epizootic was under continuous virological observation and was accompanied by a wide surveillance of feral birds Such kind of ecological experiment permitted observation of the general dynamics of the epizootic and enabled potential assessment of possible antigenic variations
The graphic display (Fig 2) shows close relatedness, almost identity (by serological criteria), between the two Yucaipa virus showing low relatedness This picture is compatible with the theories of the "common" and "serotype-specific" portions on the genomic level (Lipkind & Shihmanter, 1986) and the notions of "conserved" and "variable" regions (Nishikawa et al , 1983) and "strain-specific" epitope on the NDV HN molecule recognized by monoclonal antibodies (Iorio & Bratt, 1984) In accordance with this, one can speculate that, amongst the APMV-2 viruses, "the Yucaipa-like" PLOC virus has the largest "common" portion while the Yucaipa virus has the smallest



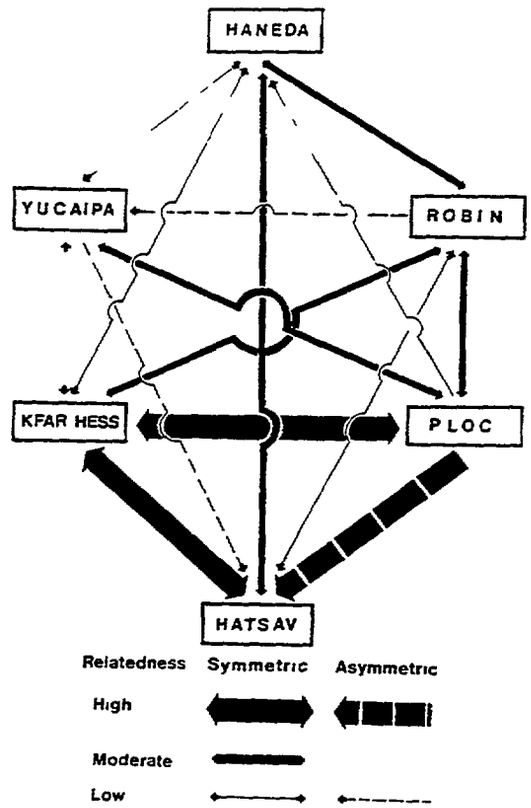


Fig 1 (left) Structural polypeptides of selected APMV-2 isolates L, HN, NP, F and M - viral proteins A and B - designations of two groups (see the text)
Fig 2 (right) Graphic display of antigenic relationships between reference and native APM-2 strains Yucaipa, Haneda, Robin and PLOC - reference APMVs, Kfar Hess (1979) and Hatsav (1980) - Israeli isolates

The following conclusions can be derived from the reported data
1 APMV-2 viruses isolated in Israel during the period 1979-81 have demonstrated wide heterogeneity by antigenicity of the HN glycoprotein, kinetics of elution and PAGE protein migration pattern with no evident correlation between the three indications
2 There is no strict correlation between the above parameters and the chronology of the virus isolations On the basis of these conclusions, two suggestions are proposed
1 During the epizootic there was a co-circulation of different variants of the APMV-2 virus varying by the above properties
2 Wide heterogeneity of the Israeli APMV-2 isolates is not the result of the evolution of one hypothetical ancestor but the viral factor causing the epizootics was heterogeneous from the very beginning

Alternative hypotheses
1 The causative factor of the epizootic was endogenous, i e the "resident" virus has undergone mutation(s) and the combination of the original virus with its mutant(s) is the cause for the observed heterogeneity
2 The causative factor of the epizootics was "imported" to Israel via migrating birds and the "imported" virus had already been heterogeneous
3 The observed heterogeneity is a result of a combination of an endogenous ("sleeping") virus with the "imported" ("alarming") virus

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CHARACTERIZATION OF NEWCASTLE DISEASE VIRUS FIELD STRAINS BY MEANS OF THREE PANELS OF MONOCLONAL ANTIBODIES ANALYSIS OF VARIATION REGULARITIES

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ABSTRACT

Seventeen reference strains and 37 recently isolated Israeli strains of Newcastle disease virus (NDV) have been studied by means of three panels of monoclonal antibodies (MAbs) against hemagglutinin-neuraminidase (HN) fusion (F) and matrix (M) viral proteins, containing 10 7 and 6 MAbs respectively The MAb-producing hybridomas were prepared against Australia-Victoria (AV) strain of NDV by fusing spleen cells from NDV-immunized BALB/c mice with the mouse myeloma cell line SP2/0 The viral envelope fraction was used for the immunization The reference strains included velogenic mesogenic and lentogenic strains isolated within 1932-1970 from domestic fowl The native NDV strains have been isolated recently (1984-1994) from various avian species including poultry (chickens and turkeys), feral (wild and show pigeons, starlings gulls eagle cattle egret), waterfowl (mallards and coots) and cage birds (parrot) The antigenic and/or functional relatedness between all the above NDV strains was examined by the pattern of their binding to each MAb, measured by ELISA technique The results revealed two contrasting indications amongst different strains a wide spectrum of variability against stable conservation in the MAb binding patterns Some of the stable strains did not differ from the homologous AV NDV although the gap between the dates of their isolations reached more than 50 years There was no correlation between the MAb binding pattern and the strain pathogenicity, host specificity geographic location and chronology of isolation Together with this there was no strong correlation between the data related to different panels for example some strains could be grouped according to the binding pattern with MAbs belonging to the HN panel but this grouping was not compatible with the results related to the other panel(s) On the background of the diverse variations some of the NDV epitopes turned out to be highly conserved

INTRODUCTION

A plenty of strains of Newcastle disease virus (NDV) isolated all over the world from various avian hosts highly differing by virulence and some other biological properties do not vary by classic serological tests Use of monoclonal antibodies (MAb) has revealed significant differences in subtle antigenic structure Topological and operational delineation of NDV proteins by means of MAb panels has found a number of nonoverlapping groups of antigenic epitopes of certain viral proteins (Iorio et al 1986, Iorio et al 1989, Russell et al 1983, Nishikawa et al 1986) which permitted to classify both reference strains and field isolates (Meulemans et al 1987, Russell et al 1990) Since the groupings were made using different MAb panels and different NDV strains, a unified mapping of NDV proteins is lacking Therefore, inclusion of new MAb panels and NDV strain sets is of importance In the present studies, three panels of MAbs against HN, F and M proteins were used for comparison of NDV reference viruses and native isolates

MATERIALS AND METHODS

Seventeen reference strains and 37 native strains of NDV isolated in Israel during the last decade from chickens (2), turkeys (2), mallards (12), wild and show pigeons (9), gulls (5), starlings (3), eagle (1), cattle egret (1), coot (1) and parrot (1) were used Hybridomas producing MAbs induced by the Australia-Victoria (AV) strain of NDV were prepared as

described (Orvell et al 1982) Indirect enzyme-linked immunosorbent assay (ELISA), Western blotting test, and antigen-capture ELISA were performed as described (Orvell et al 1982, Samson et al 1986, Li et al 1994) Radioimmunoprecipitation assay was carried out using either [^{35}S]methionine or [^{3}H]glucosamine labeling of proteins of NDV (Australia-Victoria strain) in the primary chick embryo cell monolayer culture as described (Nishikawa et al 1986) with some modifications (Umino et al 1990)

RESULTS

Ten MAbs precipitated viral protein of molecular weight (MW) 72K, and seven MAbs precipitated protein of MW 54K, both being labelled by [^{35}S]methionine as well as [^{3}H]glucosamine and corresponding to the hemagglutinin-neuraminidase (HN) and fusion (F) viral glycoproteins, respectively Six MAbs precipitated protein of MW of 36K labelled only by [^{35}S]methionine and corresponding to the viral matrix (M) protein (Fig 1) In antigen-capture ELISA test each type of MAbs was bound only to those cell lysate fractions which were precipitated with the same MAbs but not to the other two fractions In Western blot, none of the MAbs of the three panels bound to the lysate treated either by reducing agents or by high temperature (data not shown) Thus, three panels of MAbs reacting specifically with the HN, F, and M proteins, respectively, were established

All the MAbs were used for characterization of the reference strains and native field isolates of NDV In the course of these studies, a further characterization of the MAbs within each panel has been achieved The latter resulted in proving that all the MAbs belonging to the panels of anti-HN and anti-F MAbs were individually different and, hence, not relating to identical epitopes As to the anti-M MAb panel, three MAbs from 6 were indistinguishable by this criterion, i e reacted with all the 56 viruses, indicating that anti-M MAbs against at least 4 epitopes could be discerned Stability of both HN and F glycoprotein epitopes varied in wide limits as opposed to the M protein epitopes which were much more conservative

As to the viruses' characterization based on the spectrum of their binding to all the MAb panels, extremely contrasting differences amongst the viruses tested were found, varying from stable conservation to high variability For example, on one hand, there were 4 viruses which reacted with all the 23 MAbs exactly as the homologous virus AV these viruses included three reference strains isolated from chickens in 1940, 1948 and 1970 as well as an Israeli virus isolated from cattle egret in 1986 On the other hand, two Israeli field strains isolated from chickens during the same year drastically differed by the spectrum of reaction with MAbs belonging to all the three panels There were difficulties in attempts to distribute the strains by any criteria No definite correlation between the MAb binding pattern and the strain pathogenicity, host specificity, geographic location and chronology of isolation was found Together with this, there was no strong correlation between the data related to different panels

For example, some strains could be grouped according to the binding pattern with MAbs belonging to the HN panel but this grouping was not compatible with the results related to the other panel(s)

DISCUSSION

The described combination of the MAb panels against all three viral envelope proteins and 54 reference and native strains of NDV is a solid basis for the analysis of NDV antigenic epitope pattern and variability All the MAbs, except three anti-M ones, were proven to be distinct According to the Western blot test, all the MAbs appeared to be primed by conformational epitopes Similar results have been described (Samson et al 1988, Yusoff et al 1988) Among all the panels, anti-M MAbs showed a higher stability and anti-F MAbs showed a higher variability as compared to anti-HN MAbs The latter is rather in contrast to the results

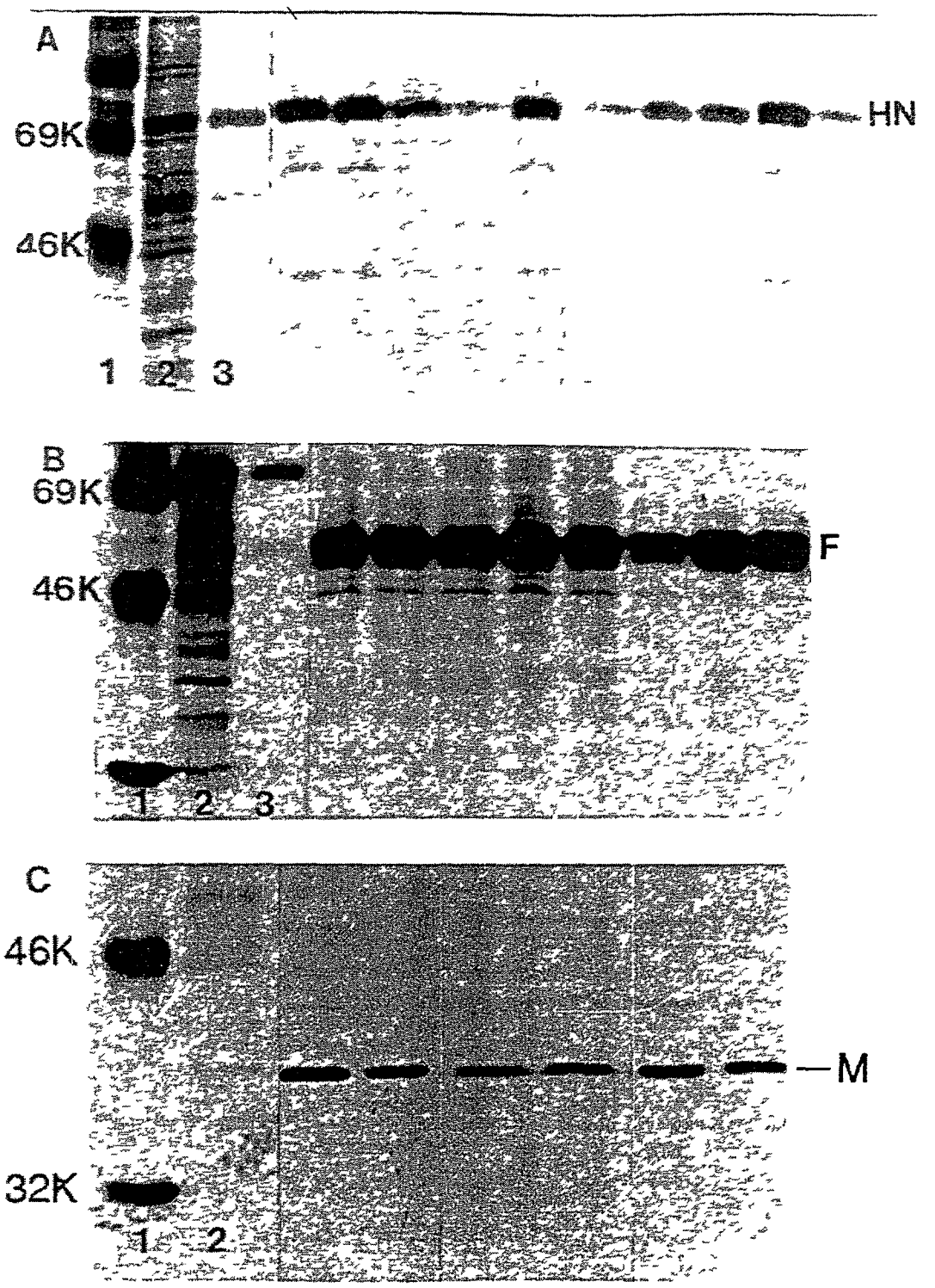

Fig 1 Radioimmunoprecipitation of NDV (AV) proteins from labelled virus-infected cell lysates by MAbs related to the following panels A Anti-HN MAbs B Anti-F MAbs C Anti-M MAbs Lane 1 MW markers Lane 2 [35S]-methionine-labelled virus-infected cell lysate Lane 3 [3H]-labelled virus-infected cell lysate All the other lanes relate to the panels of MAbs which precipitated the corresponding viral proteins from the [35S]-methionine-labelled virus-infected cell lysate

obtained in the other labs (Abenes et al , 1986, Meulemans et al 1987, Russell et al 1990) On the background of the diverse variations, some of the NDV epitopes turned out to be highly conserved

The absence of correlation between the MAb binding pattern and the strain pathogenicity, host specificity, geographic location and chronology of isolation is in accordance with the literature (Iorio et al 1986, Russell et al 1990) However, such contrasting combination of high variability with stable conservation among the NDV strains is a unique point of the present studies Namely, some of the "stable" strains did not differ from the homologous AV NDV although the time gap between their isolations is more than 50 years

ACKNOWLEDGEMENTS

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44

The geometric mean *D viviparus* burden in the group treated with moxidectin 28 days before infection was 2 (99 0 per cent reduction) and no lungworms were detected at necropsy in any of the calves treated topically with ivermectin 28 days before challenge (100 per cent reduction) The difference between the treated groups was not significant (P>0 10) and the geometric mean burdens for both groups were significantly less than the control group (P<0 01)

These results indicate that ivermectin administered by topical application and moxidectin administered by subcutaneous injection provided virtually complete protection against reinfection with *D viviparus* for at least 28 days after treatment Against *O ostertagi* the efficacy of ivermectin 28 days after treatment was >99 per cent and that of moxidectin was 86 per cent The period of sustained activity with endectocides can have practical benefits including the continued grazing of heavily contaminated pastures even where a disease outbreak has occurred (McMullin and others 1992) and the development of strategic control programmes such as the 3 8-13 programme for ivermectin (Taylor and others 1985 Armour and others 1987) Data from this study can be of use in the design of such programmes to provide optimal parasite control with minimal use of endectocides

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Antigenic characterisation of avian paramyxoviruses serotype 4 isolated from wild mallard (*Anas plathyrinchos*) in Israel

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AVIAN paramyxoviruses (APMV) apart from the well known Newcastle disease virus (NDV) include a number of other viruses which on the basis of serological tests have been placed into nine recognised serotypes (Alexander 1980 1982) Different antigenic inter relationships between the representatives of these serotypes have been found and the establishment of supergroups has been attempted (Alexander 1982 Lipkind and Shihmanter 1986 Russell and Ozdemir 1989)

The APMVs of serotype 1 (including classic NDV and APMV 1 isolated from racing pigeons) and serotype 2 (including original Yucaipa and the other Yucaipa-like viruses) are well documented and have been regularly isolated all over the world from various avian families (Gallinaceae Passeriformes Anatidae Columbidae Rallidae Ardeidae) However isolations belonging to some other serotypes are more rare so that some serotypes are represented by a prototype strain which is an initial and only isolate such as APMV 6 APMV 8 and APMV 9 The presence of different representatives of each serotype makes it possible to study the APMV s taxonomy ecology host range and evolution Therefore ecological studies aimed at revealing the occurrence of different representatives of each APMV serotype are important

Israel is located on the main flyway of birds migrating from eastern and central Europe (west branch of the flyway) and western Siberia and central Asia (east branch) to south east Africa Up to now dozens of NDVs APMV 2 (Yucaipa like) and APMV 3 viruses have been isolated in Israel from different species (Lipkind and others 1979 1981 1982a b c 1984 1985 1986 1987)

The serotype APMV 4 is represented by the prototype strain APMV-4/duck/Hong Kong/D3/75 isolated from domestic ducks in Hong Kong in 1975 (Shortridge and Alexander 1978) Since then APMV 4 viruses have been isolated predominantly from feral birds in Europe (Ottis and Bachmann 1983 Tumova and others 1984)

The first isolation of APMV 4 from feral migrating ducks in Israel and its serological identification is described here

Cloacal and tracheal swabs were taken from 36 birds shot by hunters in the northern part of the Hoola Valley Upper Galilee The swabs were propagated through chick embryonated eggs One haemagglutinating agent from tracheal and three from cloacal swabs were isolated All the isolates possessed neuraminidase activity proving they belonged to either the ortho or paramyxo virus family Double immunodiffusion tests with antiserum against influenza virus nucleoprotein antigen gave negative results Haemagglutination inhibition (HI) tests with reference anti NDV serum showed that only one of the isolates was inhibited by this serum (Table 1) The other three isolates were examined with reference antisera against the other APMV serotypes They showed positive results with antiserum against the prototype virus APMV 4/duck/3/Hong Kong/75 (Duck/3/HK) of APMV 4 serotype (Table 2) The neuraminidase inhibition test performed using the anti APMV 4 reference antiserum supported the relatedness of the above three isolates to the APMV 4 serotype (Table 3)

TABLE 1 Examination of the isolates in haemagglutination inhibition (HI) test with NDV antisera

Isolates	HI titre
NDV	480
C9	20
C25	20
C35	240
T27	40

HI titre is expressed as a reciprocal of the highest dilution of the NDV anti serum the precise calculation was done using the system of crosses according to Horsfall and Tamm (1953)

TABLE 2 Examination of the isolates in haemagglutination inhibition test with antisera against all reference APMV serotypes

Antisera against APMV serotypes	Isolates C9	C25	T27
APMV 1/NDV/Australia Victoria/32	10	10	20
APMV 2/chick/Yucaipa/California/56	<10	<10	<10
APMV 3a/turkey/Wisconsin/65	10	20	<10
APMV 3b/parakeet/Netherlands/449/77	<10	<10	<10
APMV-4/duck/Hong Kong/D3/75	160	240	320
APMV 6/duck/Hong Kong/D199/77	<10	<10	<10
APMV 7/dove/Tennessee/4/75	10	10	<10
APMV 8/goose/Delaware/1053/76	<10	<10	<10
APMV 9/duck/New York/22/80	<10	<10	<10

The same designation as that for Table 1

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TABLE 3 Examination of the isolates in neuraminidase inhibition (NI) test with APMV-4 antisera

Isolates	NI titre
APMV-4	340
C9	302
C25	240
T27	108

Neuraminidase inhibition titres are calculated according to Palmer and others (1975)

However when hyperimmune chick antisera against these isolates were prepared and used in a comprehensive cross reaction HI test some differences between the native isolates and the prototype virus were revealed (Table 4) In this experiment an original approach was used for statistical treatment (Lipkind and Shihmanter 1986 Lipkind and others 1986) Namely the value of the cross reactivity was designated as the difference (ratio) d between homologous and heterologous titres expressed in $\log_2$ The results showed different cross reactivity of the reference pro totype strain and the native isolates in relation to NDV the cross reactivity of the reference virus with NDV was symmetrical while that of the native isolates was one-way asymmetrical Further the isolates showed closer antigenic relationship to NDV than the pro totype virus There was a significant difference between the virus isolated from a tracheal swab and those isolated from cloacal swabs (Table 4)

Thus this is the first isolation of APMV 4 in Israel Antigenic comparison of the local APMV-4 isolates with the prototype strain revealed an indirect difference expressed by their different antigenic relationship towards the third virus (NDV) Such an antigenic relationship has been described in various other APMV serotypes (Lipkind and Shihmanter 1986 Lipkind and others 1986) As to the antigenic difference found between the cloaca-originated and trachea-originated isolates it may be supposed that there is a selection pressure on multiplication of hypothetically heterogeneous virus populations in respiratory and intestinal epithelial cells The phenomenon of asymmetrical cross reactivity may be an indication of substantial differences in the viral antigenic structure as has been demonstrated using a mathematical modelling approach (Lipkind and Rishe 1988 Rishe and Lipkind 1990)

TABLE 4 Haemagglutinin inhibition cross reaction test between all the isolates and APMV 1 and APMV 4 reference viruses

Antisera	Viruses NDV	APMV-4	C 9	C 25	T 27
NDV	0	3 23 ± 0 98 n = 5 P = 0 97	3 53 ± 0 91 n = 5 P = 0 97	3 35 ± 0 78 n = 5 P = 0 99	1 18 ± 0 54 n = 5 P = 0 91
APMV-4	4 58 ± 0 98 n = 3 P>0 99	0	−2 38 ± 1 12 n = 5 P = 0 91	−0 33 ± 1 26 n = 6	−1 25 ± 1 53 n = 6
C 9	0 46 ± 1 16 n = 5	0 45 ± 1 46 n = 6	0	0 91 ± 0 40 n = 6	0 04 ± 0 41 n = 5
C 25	0 01 ± 0 55 n = 5	0 03 ± 0 83 n = 5	−0 06 ± 0 69 n = 6	0	−0 63 ± 0 72 n = 6
T 27	1 10 ± 0 73 n = 5 P = 0 79	1 05 ± 1 37 n = 6	−0 18 ± 0 21 n = 6	0 80 ± 0 58 n = 6	0

Upper figure d in each cell is a mean value (with standard error) of $\log_2$ of the ratio between homologous and heterologous HI titres when a given antiserum was the same for the two compared viruses n is a number of experiments for each pair of compared viruses P is Student s coefficient of probability for the found difference d for a given number of experiments n it is expressed as a percentage of probability of the zero hypothesis ie the probability that the difference d itself differs significantly from zero The reliability of the d values (their difference from zero) was accepted as significant if it was in agreement with Fischer s 2nd criterion of probability (P = 0 95) (Lipkind and Shihmanter 1986)

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Acute encephalopathy and hyperammonaemia in a horse without evidence of liver disease

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Veterinary Record (1995) **137** 642 643

HEPATIC encephalopathy is a neurological syndrome which can accompany advanced decompensated liver disease of all types and/or extensive portal systemic shunting The condition has been recognised in horses in association with many different causes of hepatic failure including both acute and chronic liver diseases (Pearson and Craig 1980 Byars 1983 Hillyer and others 1993 Messer and Johnson 1994 Zientara and others 1994) The syn drome is associated with the toxic effects of hyperammonaemia although other substances including mercaptans gamma aminobutyric acid and short chain-fatty acids are probably also involved in the pathogenesis of encephalopathy Ammonia is pro duced in abundance in the intestinal tract especially the caecum and colon by the bacterial degradation of luminal proteins amino acids and endogenous urea This ammonia diffuses into the portal circulation and is transported to the liver where it is normally converted to urea by the Krebs Henseleit urea cycle (Dimski 1994) Hepatic encephalopathy in part reflects the failure of this important detoxification process because of extensive liver cell necrosis or direct entry of portal blood into the peripheral circula tion via portosystemic shunts This report describes the unusual

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ANTIGENIC EPITOPE CHARACTERIZATION OF MATRIX PROTEIN OF NEWCASTLE DISEASE VIRUS USING MONOCLONAL ANTIBODY APPROACH CONTRASTING VARIABILITY AMONGST NDV STRAINS

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Abstract—A panel of 15 monoclonal antibodies (MABs) against matrix (M) protein of Newcastle disease virus (NDV) was obtained and the specificity towards the M protein was proven by radioimmunoprecipitation assay and antigen capture enzyme linked immunosorbent assay (ELISA) Further studies were directed to antigenic epitope mapping of the M protein by means of this panel The epitope characterization was performed by competitive antibody-binding assay by means of labelling each MAB with biotin [3] At least three clear non overlapping and two partially overlapping groups were determined each including four one eight one and one MAB respectively All the above MABs appeared to be induced by structural epitopes formed in conditions of tertiary structure of the native M antigen Twelve reference and 51 recently isolated local NDV strains have been studied by means of this MAB panel several lineages having been revealed The high stability of some epitopes and different variability of the others was demonstrated No correlation between the above lineages and some other properties of the studied NDV strains (host specificity date and place of isolation) has been found © 1997 Elsevier Science Ltd

Key words Newcastle disease virus matrix protein antigenic epitopes monoclonal antibodies radioimmunoprecipitation assay

Resume—Un panel de 15 anticorps monoclonaux (MAB) contre la proteine M du NDV a ete obtenu et leur specificite contre la proteine M demontree par radio immuno precipitation et par ELISA capture Des travaux supplementaires ont ete effectues pour obtenir une carte epitopique de la proteine M en utilisant ce panel La caracterisation epitopique a ete faite par essai competitif d attachement des anticorps par marquage a la biotine de chacun des MAB Au moins trois groupes independants et deux groupes avec un recouvrement partiel one ete determines chacun comprenant quatre un huit un et un MAB Tous ces MAB se sont reveles induits par des epitopes de structure formes dans des conditions de structure tertiaires de l antigene M natif Douze souches locales isolees recemment de NDV ont ete etudiees par ce panel d anticorps monoclonaux La grande stabilite de certains epitopes et la variabilite relative des autres a ete demontree Aucune correlation entre les proprietes de ces lignees et celles des souches NDV n a pu etre trouvee © 1997 Elsevier Science Ltd

Mots clefs Virus de la maladie de Newcastle matrix proteine epitopes antigeniques anticorps monoclonaux radio immuno precipitation

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47

INTRODUCTION

Matrix (M) protein the smallest (34–40 kDa) of six structural proteins of paramyxoviruses, forms an internal viral envelope embracing viral nucleocapsid and adjoining it to the internal part of the viral envelope [4] The primary structure of the M protein was deduced from the nucleotide sequence of the corresponding M gene that has been determined for certain paramyxoviruses [5–7], including Newcastle disease virus (NDV) [8 9] These studies revealed the remarkable genetic stability of the M protein

Although there is no evidence for any enzymatic activity of the M protein its role in the assembly of different viral components has been demonstrated [10] The central localization of the M protein between the integral envelope of glycoproteins and the internal core proteins, on the one hand and its crucial role in virus assembly realized via physicochemical connections with the other viral proteins on the other hand, suggest a complicated (for a rather small protein) structural arrangement providing different functional activities Within a virion, the M protein molecules are associated with lipid membranes [11–14] envelope glycoproteins [15–17] and nucleocapsid [18], in addition there is a strong predilection for association of the M-protein molecules with each other [18 19] In contrast to other paramyxoviral proteins which accumulate only in cytoplasm of the infected cells, the M protein of the NDV has also been detected in the nucleus [20–22] These different functional activities must be realized via particular M protein active sites responsible for specific coupling between the corresponding loci in the envelope and core viral proteins, and certain specific intracellular sites [22]

It is reasonable to expect that this functional burden associated with the structural organization of the M protein is connected to its antigenic pattern which could be revealed via MAB-mediated analysis In this respect, however, different results have been described Two non-overlapping sites, one of them including three partially overlapping epitopes have been obtained using a panel of eight MABs against D26 strain of NDV [23] Another group of authors [21], using a panel of five MABs against Australia–Victoria NDV strain, has found three non-overlapping sites A third group of workers using a panel of six MABs against Ulster NDV strain has described three partially overlapping antigenic sites on the M protein of the Ulster NDV strain [24]

In contrast to the envelope glycoproteins, the M protein and core proteins of the NDV are not exposed to significant immunological pressure [25] Antibodies against these proteins possess neither virus-neutralizing nor protective capacities both *in vitro* and *in vivo* and do not inhibit significantly any known functional viral activities [24 26] At the same time antigenic variations of the M protein of different NDV strains have been revealed using panels of anti-M MABs [21 23] In the present studies a panel of 15 anti-M MABs extended antigenic characterization of the M protein by detailed epitope mapping and by using a considerably larger library of NDV strains including conventional reference strains as well as local isolations from domestic and feral birds

MATERIALS AND METHODS

Reference viruses

The Australia–Victoria (1932) strain of NDV (obtained from M A Bratt University of Massachusetts, Worcester MA, U S A) was used for the production of MABs The other reference NDV strains (Herts, Komarov, Texas, Mukteswar Italien LaSota Hitchner, Roakin Warwick Queensland, Eastwood) were obtained from D J Alexander

48

(Central Veterinary Laboratory, Weybridge, U K) Propagation of the viruses was performed as described earlier [27]

NDV local field isolates

Fifty-one NDV strains have been isolated in Israel during the last decade (1984–1994) from both domestic and feral birds The former included chickens (3 isolates), turkeys (6) and Chinese ducks (1) The feral birds included waterfowl, like mallards (15) and coots (2), sea birds, like gulls (5), passerine birds, like starlings (3), cage birds peacock (1), parrot (1) and love-bird (1), and others pigeons (11), cattle egret (1) and eagle (1) Most of the viruses were isolated from cloacal and/or tracheal swabs, in some cases *post mortem* material was used for isolation The field samples treated by antibiotics were inoculated into the allantoic cavity of the 10–11 day-old chick-embryonated eggs, which were incubated until embryo death or until the period of 6 days' post-inoculation The haemagglutinating (HA) agents serologically identified as NDV had undergone a minimal number of passages (usually one to two) through embryonated eggs in order to accumulate an amount of virus of high titre for a virus stock stored at −70°C The virus purification was carried out by differential centrifugation through sucrose density gradients [28] Protein quantitation was done using Coomassie Protein Assay Reagent (PIERCE)

Hybridoma production and MAB screening

MABs were prepared by fusing spleen cells from NDV-immunized BALB/c mice with the mouse myeloma cell line SP2/0 The viral envelope fraction obtained by *n*-octyl-D-glycoside treatment of the purified virus preparation and containing haemagglutinin-neuraminidase (HN), fusion (F) and the M viral proteins was used for the immunization of mice as described previously [1, 2] Screening of the MAB-secreting hybridoma clones was performed by indirect enzyme-linked immunosorbent assay (ELISA) using purified virus as antigen and haemagglutination inhibition test as described previously [2] Selected MAB-secreting hybridoma clones were inoculated into pristane-primed BALB/c mice Ascitic fluids were collected 1–2 weeks later

MAB characterization

The MABs were characterized for specificity by radioimmunoprecipitation assay (RIPA) using labelling of viral proteins with [^{35}S]methionine, immunoblot analysis (IB) and two-antibody sandwich ELISA (TAS–ELISA) RIPA were performed as described previously [2] IB was carried out as described by Samson [29] The TAS–ELISA was performed as described by Li *et al* [30], with some modifications Accordingly an anti-M MAB of high avidity was chosen, purified by means of the Kit PURE (Sigma Immunochemicals St Louis, MO U S A) and used for coating Dynateck plates at a concentration of 20 μg/ml After incubation for 2 h in a humid atmosphere and washing the blocking buffer containing neutral bovine serum albumin (3%) Tween 20 (0 05%) and PBS was added After a 2 h incubation followed by washing with 0 05% Tween–PBS solution, 50 μl per well of the antigen (viral lysate) solution in the blocking buffer was added After incubation at 37°C for 1 h and washing, the sandwich ELISA was used for MAB detection The MABs to be detected were labelled with biotin and added to the sandwich ELISA in two-fold dilutions After 45 min incubation at 37°C and washing, the ExtrAvidin TM–peroxydase solution (BioMakor, Rehovot, Israel), prepared according to the kit recommendation, was added The further steps were performed as described previously [2]

Competitive binding assay

Competitive binding assay was carried out according to Nishikawa *et al* [23] with some modifications, namely, the use of biotin-labelled (instead of peroxydase-labelled) MAB and, correspondingly, ExtrAvidin TM–peroxydase [3]

Determination of MAB isotype

Isotypes of the monoclonal antibodies were determined by Ouchterlony immunodiffusion test using Mouse Monoclonal Typing Kit (The Binding Site, Birmingham, U K)

RESULTS

Characterization of the MAB panel

The MAB panel was first characterized by means of ELISA (Table 1) It can be seen that five MABs (M3b, M3d, M3e, M3f and M3g) had a titre of 10^7 The titres of the other 10 MABs were 10^3 By the isotype examination, all of them fell into the same immunoglobulin (Ig)-G class the majority being related to the IgG1 subclass

The MABs were examined for their specificity towards M protein by means of three tests RIPA (Fig 1) TAS–ELISA (Table 1) and IB (Table 1) Both the RIPA and the TAS–ELISA demonstrated clearly that all the MABs were directed against M protein It should be noted that all the MABs were negative in immunoblots and in the haemagglutination inhibition test (Table 1)

Antigenic mapping of the M protein

Antigenic mapping of the M protein was carried out by reciprocal competitive binding assay In preliminary experiments, saturating doses of the MAB were determined as described by Nishikawa *et al* [32]

The general picture has revealed at least five antigenic sites (Fig 2) which can be arranged into two groups corresponding to the antigenic regions upon the M protein (Fig 5) The first region (A) includes two non-overlapping sites (sites 1 and 2) and one

Table 1 Immunological properties of the MAB to NDV strain Australia Victoria

				Two antibody sandwich ELISA				
MAB	Class/subclass	HI titre	ELISA	M	HN	F	IB	RIPA
M1a	IgG1	<8	+3	+2			neg	M
M1b	IgG1	<8	+3	+3			neg	M
M1c	IgG2a	<8	+3	+3			neg	M
M1d	IgG1	<8	+3	+3			neg	M
M1 2a	IgG1	<8	+3	+3			neg	M
M2a	IgG1	<8	+3	+2			neg	M
M3a	IgG2a	<8	+3	+2			neg	M
M3b	IgG1	<8	+2	+3			neg	M
M3c	IgG2a	<8	+3	+2			neg	M
M3d	IgG1	<8	+2	+2			neg	M
M3e	IgG1	<8	+2	+2			neg	M
M3f	IgG1	<8	+2	+2			neg	M
M3g	IgG1	<8	+2	+2			neg	M
M3h	IgG2a	<8	+3	+2			neg	M
M4a	IgG1	<8	+3	+3			neg	M

Class/subclass of MABs were determined by Mouse Monoclonal Typing Kit (RK008 The Binding Site)
HI titre Haemagglutination inhibition test performed as described previously [27]
IB Immunoblot, ELISA and two antibody sandwich ELISA were performed as described in Materials and Methods

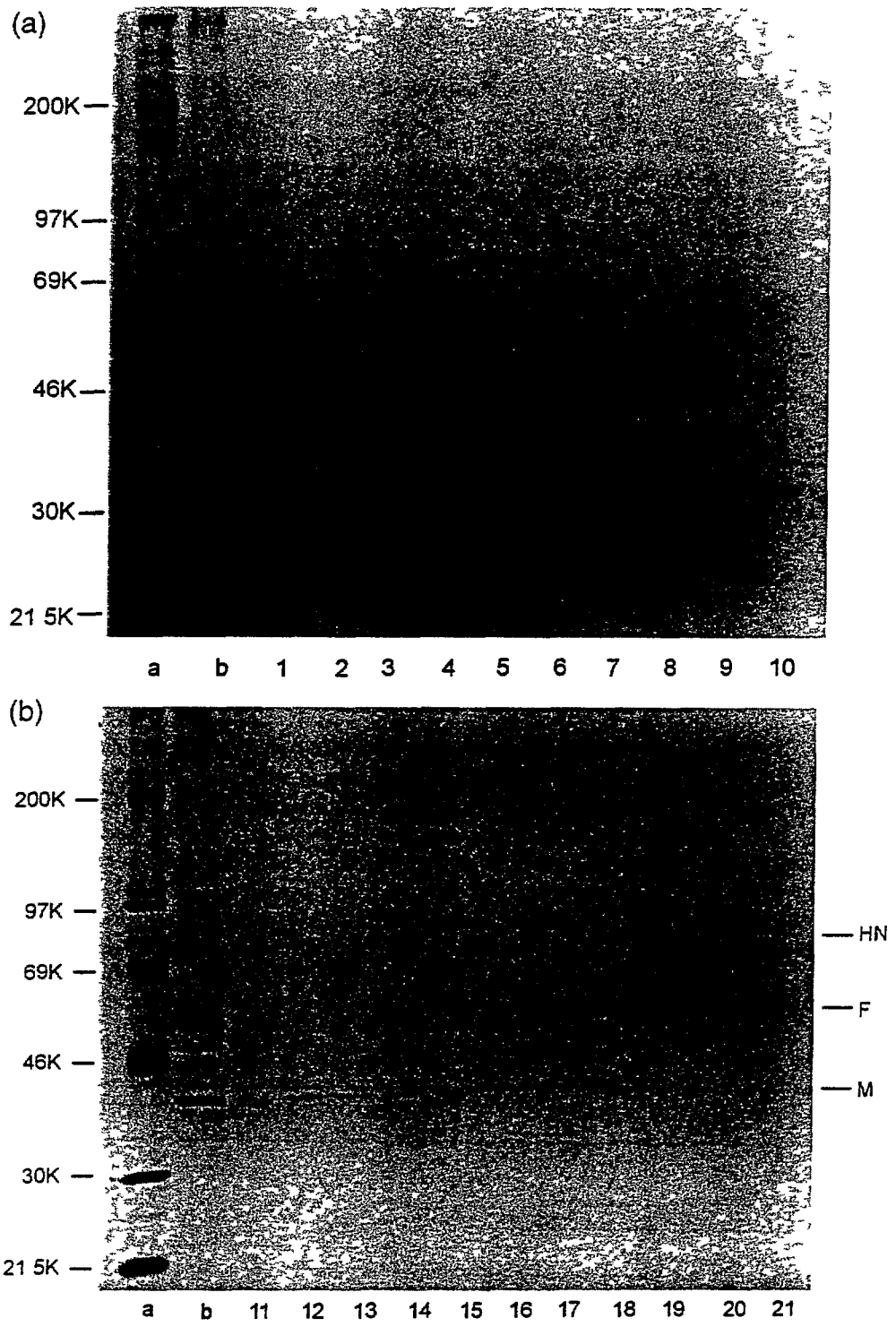

Fig 1 Radioimmunoprecipitation of NDV viral proteins by MABs [^{35}S] labelled virus lysates were subjected to immunoprecipitation with MABs the immune complexes were collected on protein A bacterial adsorbent (Sigma) electrophoresed and autoradiographed Immunoprecipitates of MABs were electrophoresed in lanes numbered as follows M1a M1b M1c M1d M1 2a M2a M3a M3b M3c M3d M3e M3f M3g M3h M4a (lanes 1–15 respectively) reference anti M MABs (lanes 16 17 and 18) anti-HN MAB (lane 19) anti-F MAB (lane 20) control mouse ascites fluid [BioMakor] (lane 21) MW markers (lane a) and [^{35}S]-methionine labelled purified virus (lane b)

site (site 1 2) overlapping with sites 1 and 2 The second region (B) includes two asymmetrically overlapping sites (sites 3 and 4) Asymmetric overlapping here means that the MAB against site 4 inhibits the binding of three MABs to site 3 while the MABs against site 3 do not inhibit MAB binding to site 4

Four MABs specific to site 1 (M1a, M1b M1c and M1d) and one MAB specific to site 1 2 (M1 2a) competed with each other but did not compete with the rest of the MABs In addition, the MAB M1 2a competed with all the MABs specific to the site 1 as well as with the MAB M2a specific to site 2 while MAB M2a competed only with MAB M1 2a

Eight MABs were specific to site 3 From these, seven MABs (M3a, M3c M3d, M3e M3f M3g and M3h) showed a certain asymmetry in the cross-reactivity with each other in reciprocal competition test (Fig 2), while MAB M3b did not show any asymmetry

The MAB M4a (directed against site 4 of M protein) was distinctive from the other MABs, namely, the labelled MAB M4a was not blocked by any other MABs of the panel while this MAB itself significantly inhibited (by more than 60%) the capacity of the MABs M3e M3f, M3g and M3h to bind to the antigen Moreover, even more than a 100-fold excess of the concentration of the unlabelled MAB had no effect, the competitive inhibition did not exceed 14% (data are not shown)

Antigenic structure of the M protein of different NDV strains

The above MAB panel was used for antigenic characterization by ELISA of 62 NDV heterologous strains, including 11 reference strains and 51 field isolates As can be seen in Figs 3 and 4, two antigenic sites, site 1 and site 4, are remarkably conserved Namely, three (M1b, M1c, and M1d) of the four MABs specific to site 1 and MAB M4a, the only one specific to site 4, reacted with all the studied NDV strains Of the four MABs specific to site 1, only MAB M1a showed a negative reaction with four of the 62 viruses

MABs specific to site 3 showed diverse patterns On one hand, the epitope M3g was remarkably conserved like the above conserved epitopes of sites 1 and 4 In contrast three epitopes, M3a, M3f and M3h showed definite variability These MABs showed negative reactions with 35–45 out of the 51 native isolates and with 3–4 out of the 11 reference viruses The other four epitopes, M3e, M3d, M3b and M3c, displayed intermediate patterns Namely towards the reference viruses, the respective specific MABs

Competing antibody	Biotinylated antibody															Antigenic site
	M1a	M1b	M1c	M1d	M1 2a	M2a	M3a	M3b	M3c	M3d	M3e	m3f	M3g	M3h	M4a	
M1a	+	+	+	+	+	+/										1
M1b	+	+	+	+	+											1
M1c	+	+	+	+	+	+/										1
M1d	+	+	+	+	+											1
M1 2a	+	+	+	+	+	+										1 2
M2a					+	+										2
M3a							+	+	+	+	+	+	+	+		3
M3b							+	+	+	+	+	+	+	+		3
M3c							+	+	+	+	+/	+/	+	+		3
M3d							+/	+	+	+	+	+	+	+/		3
M3e							+/	+	+	+	+	+	+	+/		3
M3f							+/	+	+	+	+	+	+	+		3
M3g							+/	+	+	+	+	+	+	+		3
M3h							+	+	+	+	+	+	+	+		3
M4a							+/		+/	+/	+	+	+	+	+	4

Fig 2 Epitope assignments of anti M MABs [+] > 60% competition [±] 50–60% competition [ ] < 50% competition

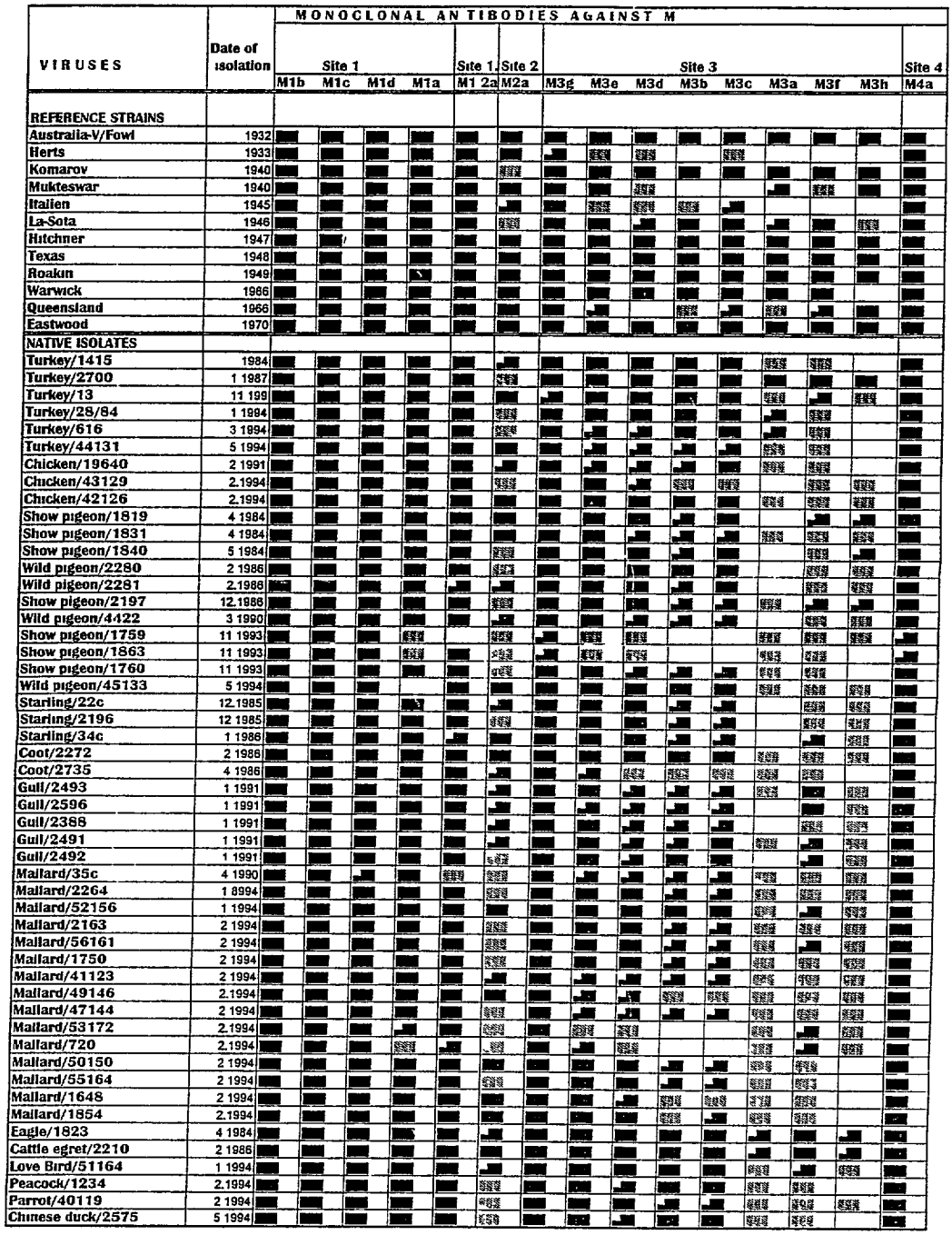

Fig 3 Reactivities of MABs with the reference strains and native field isolates of NDV Reactivity of each MAB with different NDV strains is expressed as a percentage of their binding (measured by ELISA) to the respective strains in relation to that of the homologous (AV) strain The values represent the average of three separate experiments Ascites fluids were used as the sources of antibodies ■ > 50% of MAB binding to the antigen of a heterologous virus related to that of a homologous virus ▬ 30–50% of the binding ▒-10–30% of the binding □- < 10% of the binding The values of 30% or more are considered to indicate positive binding the values less than 30% are considered as negative binding

showed the same pattern as the above-described MAB specific to the 'variable epitopes while, towards the native isolates, these MABs were closer to those which were specific to the conserved epitopes

As for site 2, there was only one MAB (M2a) which was similar to the MAB specific to the variable' epitopes In addition, there was one MAB (M1 2a) specific to the site which according to the overlapping results (Fig 2), occupied an intermediate position between site 1 and site 2 Thus, the corresponding epitope appeared to be conserved

DISCUSSION

The panel used in the present study includes 15 MABs, i e more than any other of the panels of the M protein-specific MABs that have been described previously [21, 23, 24] By using the avidin–biotin system instead of the commonly used peroxydase technique, a higher sensitivity for the competitive blocking test was achieved This provided a clearer determination of the M-protein antigenic epitopes and a better distinction between the antigenic sites

The competitive blocking test revealed two non-overlapping regions The first one included two non-overlapping sites (site 1 and site 2) and one site (site 1 2) that overlapped partially with both site 1 and the site 2 [Fig 5 (A)] This situation, when there are two non-overlapping sites but there is an 'intermediate' site that overlaps with either of these non-overlapping sites, has been described by Iorio *et al* [31] for a panel of MABs against the HN protein The second region included two overlapping sites [Fig 5(B)] site 3 represented by eight epitopes and site 4 represented by one epitope

There is a certain hierarchical difference between our classification and that by Nishikawa *et al* [23] The latter considers two 'major non-overlapping antigenic sites' the first corresponds to a group of rather homogeneous MABs while the second was

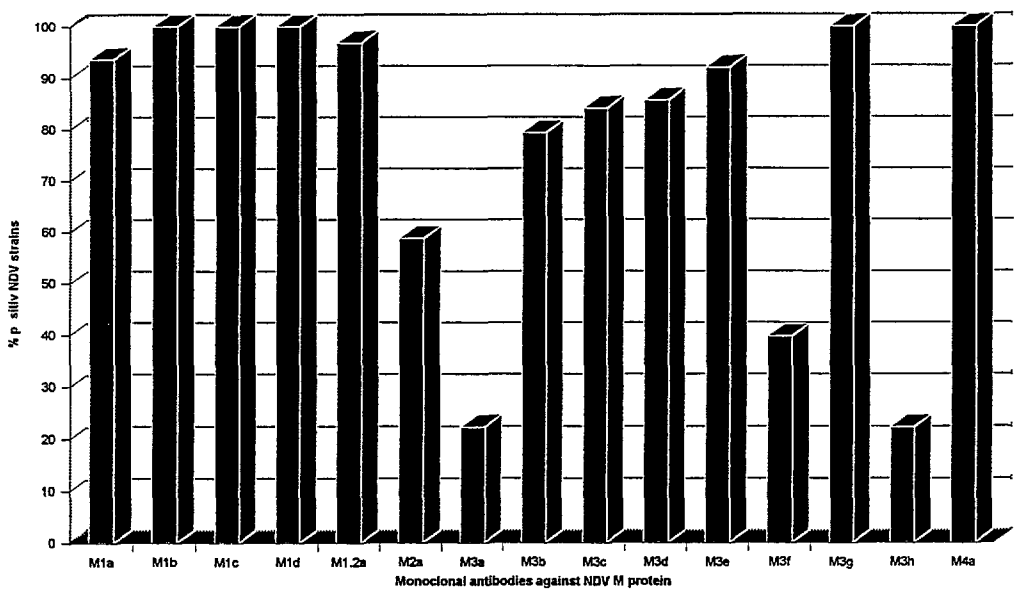

Fig 4 Relative (percentage) number of NDV strains recognized by particular MABs

54

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56

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ANTIGENIC HETEROGENEITY OF AVIAN PARAMYXOVIRUSES OF SEROTYPE 2 ("YUCAIPA-LIKE") ISOLATED FROM DOMESTIC AND WILD BIRDS IN ISRAEL

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Abstract—Thirty three viruses of PMV 2 serotype isolated in Israel from domestic and wild birds during epizootv of a respiratory disease in 1979–1981 were studied comprehensively in comparison with a set of reference PMV 2 viruses using cross reaction hemagglutination inhibition tests elution–hemagglutination pattern and pattern of migration of viral proteins in polyacrylamide gel electrophoresis The results demonstrated considerable heterogeneitv amongst the local isolates bv the above three criteria which were not correlated with each other There was no significant correlation between the differences found and chronology of isolation of the viruses The results indicated that there mav have been simultaneous co circulation of different PMV 2 strains in the local avian reservoir at the beginning of the epizootic rather than temporal accumulation of variants as a result of antigenic drift of an ancestor virus which caused the initial infection

Key words Yucaipa virus antigenicity hemagglutination–elution viral proteins ecology

Resume—Trente trois souches de virus serotype PMV 2 isolees d oiseaux domestiques et sauvages durant une epizootie de maladie respiratoire pendant la periode 1979–1981 ont ete etudiees comparativement avec un panel de souches PMV 2 de reference par l inhibition croisee de l hemagglutination le modele d elution–hemagglutination et la migration des proteines virales dans polyacrylamide gel electrophorese Une heterogeneite marquee a ete demontree parmi les souches locales selon les criteres examines ne montrant aucune relation avec la chronologie de l isolation des virus Les resultats obtenus indiquent la possibilite d une co circulation simultanee des differents virus PMV 2 dans le reservoir aviaire local des le commencement de l epizootie plutot qu une accumulation graduee de variants due a des changements antigeniques d un virus ancestral qui fut l initiateur de l infection

Mots clefs Yucaipa virus antigenicite hemagglutination–elution proteines virales ecologie

INTRODUCTION

In recent years there have been numerous isolations of paramyxoviruses (PMV) from a variety of avian species [2–4] and viruses from 4 of these serotypes have been shown to affect turkeys PMV-1 (NDV), PMV-2 (Yucaipa-like), PMV-3 and PMV-6 [4] Viruses

57

of the PMV-2 serotype serologically related to the first isolate PMV-2/chicken/California/Yucaipa/56 [8], have been shown to be widespread among turkeys in North America [2] Viruses of this subtype have also been isolated from wild and captive birds particularly, passerine species, in most major areas of the world [2–4, 9, 12–15, 20 28, 32]

Up to 1979, no avian paramyxovirus other than NDV had been isolated from birds in Israel Systematic surveillance of wild and domestic birds for influenza viruses began in 1978 and, as a result of those studies, a PMV-2 virus was isolated in January 1979 from mallard ducks (*Anas platyrhynchos*) migrating into Israel [26] During the winter of 1978–79 a series of respiratory disease problems occurred on turkey farms in Israel and appeared to spread The disease was characterized by signs ranging from mild rales to severe respiratory distress including conjunctivitis, nasal discharge, sinusitis and pneumonia, and egg production was severely affected in breeder flocks Morbidity usually approached 100% while mortality varied from 5 to 90% [23] In March 1979 several hemagglutinating (HA) agents isolated from outbreaks of the disease were identified as avian PMV of the PMV-2 serotype Since that time investigation of the disease in turkeys and surveillance of other birds has resulted in the isolation of more than 70 PMV-2 viruses from all parts of Israel [27] However experimental infection of turkeys by the isolated Yucaipa virus did not cause clear clinical symptoms unless the infection by the Yucaipa virus was combined with some bacterial or mycoplasma agents which were also isolated at that time from affected turkey flocks [16]

The majority of the isolates were obtained from turkeys, but isolations have also been made from domestic fowl [27], coots (*Fulica atra*) [25] and cattle egret (*Ardeola ibis*) [24]

It was the object of the work described in the present paper to compare characteristics of a selection of the PMV-2 isolates from birds in Israel in order to detect any variations among the isolates

MATERIALS AND METHODS

Viruses

Well-characterized PMV-2 viruses were used as reference viruses and these are listed in Table 1 Field isolates were obtained as described [25–27] Thirty three isolates were examined and these are also listed in Table 1

All the viruses were propagated in embryonated fowls eggs as described [23]

Serological tests

Hemagglutination (HA) and hemagglutination inhibition (HI) tests were done as described [23] Antisera were prepared in chickens as described by Alexander and Collins [5] The inoculation of 5 chickens was carried out in an isolation box and the sera obtained after booster were pooled Field sera from sick turkeys were also used

Statistical treatment of the data of HI tests was performed as described [21 22] The treatment was performed because of considerable fluctuations in the numerical data of HI titers in various experiments It was found, however, that the fluctuations in the corresponding values for cross reactivity (differences between the values of homologous and heterologous titers) were substantially less Therefore, the value of the cross reactivity designated as a difference d between homologous and heterologous titers expressed as $\log_2$, was used for statistical treatment based on the t-test [38] The Student's probability

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of the reference PMV 2 strains and the local PMV 2 viruses isolated in Israel which were used in the s udies

Viruses	Abbreviations
Reference Strains	
2/chicken/California/Yucaipa/56	Yucaipa
2/finch/North Ireland/Bangor/73	Bangor
2/robin/Hiddensee/19/75	Robin
2/cut throat weaver/Senegal/PLOC 9/76	PLOC
2/mannikin/Japan/Haneda 35/76	Haneda
2/finch/England/347/76	England
2/turkey/Italy/F/77	Italy
2/*Sturnus contra*/Indonesia/3589/79	Indonesia/3589
2/*Sturnus contra*/Indonesia/3650/79	Indonesia/3650
2/gadwall/Kenya/80	Kenya
Israeli Isolates	
V 2/Mallard/Israel Ramon/4/T/79 1	Mal/Ramon
2/Turkey/Israel Kfar Hess/7/T/79 3	Ty/Kfar Hess/7
V 2/Turkey/Israel Kfar Hess/10A/B/79 3	Ty/Kfar Hess/10A
V 2/Turkey/Israel Kfar Hess/10B/B/79 3	Ty/Kfar Hess/10B
V 2/Turkey/Israel Burgata/116/B/79 4	Ty/Burgata
V 2/Turkey/Israel Beit Shearim/170/L/79 4	Ty/Beit Shearim/170
V 2/Turkey/Israel Sde Yoav/15/T/79 9	Ty/Sde Yoav/15/T
V 2/Turkey/Israel Sde Yoav/15/C/79 9	Ty/Sde Yoav/15/C
V 2/Cattle egret/Israel, Ramat Gan/9/T/80 9	C egret/Ramat Gan
V-2/Turkey/Israel Beit Herut/584/B/80 10	Ty/Beit Herut
V 2/Chicken/Israel Mishmeret/589/L/80 10	Ch/Mishmeret/589
2/Turkey/Israel Neot Mordehai/593/80 10	Ty/Neot Mordehai
2/Turkey/Israel, Beit Shearim/595/80 10	Ty/Beit Shearim/595
V 2/Turkey/Israel Rosh Tsurim/596/80 10	Ty/Rosh Tsurim
PMV 2/Turkey/Israel Bear Tuvia/676/B/80 10	Ty/Bear Tuvia/676
PMV 2/Turkey/Israel Kfar Harutz/685/B/80 10	Ty/Kfar Harutz
PMV 2/Turkey/Israel Nahla/687/B/80 10	Ty/Nahla/687
PMV 2/Chicken/Israel Hatsav/734/B/80 11	Ch/Hatsav
PMV 2/Turkey/Israel Bear Tuvia/752/B/80 11	Ty/Bear Tuvia/752
PMV 2/Chicken/Israel Mishmeret/803/K/80 11	Ch/Mishmeret/803
PMV 2/Turkey/Israel Shalva/810/B 80/11	Ty/Shalva
PMV 2/Turkey/Israel Nahla/812/B/80 11	Ty/Nahla 812
PMV 2/Turkey/Israel Sde Moshe/813/B/80 11	Ty/Sde Moshe
PMV 2/Coot Israel Shluhot/868/S/80 12	Coot Shluhot
PMV 2/Chicken/Israel Petahia/817/B/81 1	Ch/Petahia
PMV 2/Turkey/Israel Ein Tsurim/818/B/81 1	Ty/Ein Tsurim
PMV 2/Turkey/Israel Tsora/830/B 81 1	Ty/Tsora
PMV 2/Turkey/Israel Talmi Ehiel/837/B/81 1	Ty/Talmi Ehiel
PMV 2/Turkey/Israel Beit Yitzhak/846/C/81 1	Ty/Beit Yitzhak
PMV 2/Turkey/Israel Hatsav/4/T/81 1	Ty/Hatsav/4/T
PMV 2/Turkey/Israel Hatsav/6/T/81 3	Ty/Hatsav/6/T
PMV 2/Coot/Israel Beit Alfa/5/T/81 3	Coot/Beit Alfa
PMV 2/Turkey/Israel Hatsav/8/T/81 6	Ty/Hatsav/8/T

or the differences d for a given number of experiments n was calculated The of the d values (their difference from zero) was accepted as significant if it was ment with Fischer s second criterion of probability (95% probability $P = 0\ 95$) principles of statistical treatment [38], were applied to two-sided asymmetric ctivity (cases displaying one-sided asymmetry evidently did not need any statistical) In this case, the cross reactivity between two viruses X and Y was considered etric if the difference d_1 (when anti-X serum was used against both the viruses) antly different from d_2 (when anti-Y serum was used against both the viruses) e estimation of the difference by the d indication was as follows $d \leqslant 1$—low e, $1 \leqslant d \leqslant 2$—moderate difference, $d > 2$—high difference

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Structural polypeptide analysis

Structural polypeptides were analyzed by polyacrylamide gel electrophoresis under reducing conditions in the presence of sodium dodecylsulfate (SDS–PAGE) as described by Alexander and Collins [5]

Virus elution from red blood cell

Elution of the viruses from RBC was estimated as described [37] According to the estimation, the viruses were placed into 4 groups as follows *Non-eluters* which showed no elution after 24 h *Partial eluters* which showed only incomplete elution at 24 h *Moderate eluters* showing no elution or incomplete elution at 4 h but full elution at 24 h *Fast eluters* which showed full elution within 4 h

RESULTS

HI tests with reference PMV-2 viruses

A large number of ' Yucaipa-like viruses have been described up to now [14] and it was considered important to investigate the antigenic relationships between these ' reference viruses to enable assessment of the significance of differences that may be detected within the Israeli isolates Ten reference and 2 Israeli PMV-2 viruses and antisera against 4 of the reference (Yucaipa Robin, PLOC, and Haneda), and 2 Israeli strains, which included the first isolate made at the beginning of the epizooty in April 1979 (Ty/Kfar-Hess/10B), and one isolated at the peak of the epizooty in November 1980 (Ch/Hatsav) were used in these experiments

It can be seen (Table 2), that the 2 selected Israeli isolates did not differ from each other by the criterion of cross reactivity

$$\frac{\text{Ty/Kfar-Hess/10B}}{\text{Ch/Hatsav}} = \frac{0\ 75 \pm 0\ 68}{0\ 17 \pm 0\ 87}$$

All the reference viruses were inhibited at high levels by each serum and the degree of the cross reactivity varied considerably, so that amongst these strains no one strain was similar to another For example for Yucaipa, Robin, PLOC and Haneda viruses, whose antisera were also used in the tests and hence, the two-sided cross reactivity could be determined and quantitated, the pairwise comparison showed no similarity between the 4 strains In the case of two pairs Yucaipa–Robin and PLOC–Haneda one-sided asymmetric cross reactivity was observed when anti-Yucaipa serum against Robin and anti-PLOC antiserum against Haneda were used there was no difference between homologous and heterologous HI titers (d is close to zero) while when anti-Robin serum against Yucaipa and anti-Haneda serum against PLOC were used then the difference d was more than 3 $\log_2$ in the former case and more than 2 $\log_2$ in the latter one, namely

$$\frac{\text{Yucaipa}}{\text{Robin}} = \frac{0\ 13 \pm 0\ 24}{3\ 38 \pm 0\ 46}$$

and

$$\frac{\text{PLOC}}{\text{Haneda}} = \frac{0\ 32 \pm 0\ 27}{2\ 38 \pm 0\ 20}$$

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Table 2 Cross HI test with reference NDV strains

Antisera	Viruses: Yucaipa	Robin	PLOC	Haneda	Bangor	England	Italy	Indonesia 3589	Indonesia 3650	Kenya	Ty/ Kfar Hess 10B	Ch/ Hatsav
Yucaipa	0	0.13 ± 0.24 $n = 4$ $P = 0.35$	1.37 ± 0.17 $n = 6$ $P > 0.99$	1.02 ± 0.21 $n = 9$ $P > 0.99$	1.59 ± 0.51 $n = 4$ $P = 0.95$	0.96 ± 0.74 $n = 4$ $P = 0.72$	0.94 ± 0.35 $n = 4$ $P = 0.93$	0.34 ± 0.17 $n = 3$ $P = 0.82$	0.39 ± 0.39 $n = 3$ $P = 0.58$	1.19 ± 0.21 $n = 4$ $P = 0.99$	0.89 ± 0.24 $n = 11$ $P = 0.99$	0.46 ± 0.23 $n = 6$ $P = 0.90$
Robin	3.38 ± 0.46 $n = 4$ $P > 0.99$	0	2.01 ± 0.01 $n = 2$ $P > 0.99$	1.89 ± 0.45 $n = 3$ $P = 0.95$	2.22 ± 0.35 $n = 3$ $P > 0.96$	2.57 ± 0.30 $n = 3$ $P > 0.96$	1.63 ± 0.39 $n = 3$ $P = 0.95$	2.06 ± 0.12 $n = 3$ $P > 0.96$	1.35 ± 0.32 $n = 3$ $P = 0.95$	3.23 ± 0.45 $n = 3$ $P > 0.99$	1.73 ± 0.71 $n = 8$ $P = 0.95$	2.03 ± 0.57 $n = 4$ $P = 0.96$
PLOC	1.88 ± 0.30 $n = 6$ $P > 0.99$	0.95 ± 0.76 $n = 3$ $P = 0.65$	0	0.32 ± 0.27 $n = 7$ $P = 0.72$	0.66 ± 0.17 $n = 4$ $P = 0.97$	0.62 ± 0.33 $n = 3$ $P = 0.82$	0.54 ± 0.52 $n = 4$ $P = 0.61$	0.08 ± 0.23 $n = 4$ $P = 0.22$	−0.26 ± 0.91 $n = 3$ $P = 0.21$	1.31 ± 0.63 $n = 3$ $P = 0.83$	0.48 ± 0.50 $n = 10$ $P = 0.66$	0.19 ± 0.50 $n = 6$ $P = 0.29$
Haneda	2.54 ± 0.29 $n = 9$ $P > 0.99$	1.68 ± 0.29 $n = 3$ $P = 0.97$	2.38 ± 0.20 $n = 7$ $P > 0.99$	0	2.40 ± 0.56 $n = 4$ $P = 0.98$	1.44 ± 0.22 $n = 3$ $P = 0.98$	1.42 ± 0.20 $n = 4$ $P > 0.99$	1.01 ± 0.17 $n = 3$ $P = 0.99$	0.79 ± 0.83 $n = 4$ $P = 0.54$	2.50 ± 0.21 $n = 4$ $P > 0.99$	2.02 ± 0.18 $n = 12$ $P > 0.99$	1.50 ± 0.24 $n = 8$ $P > 0.99$
Ty/Kfar Hess 10B	2.06 ± 0.51 $n = 9$ $P > 0.99$	1.28 ± 0.47 $n = 5$ $P = 0.95$	1.21 ± 0.68 $n = 8$ $P = 0.88$	1.01 ± 0.59 $n = 9$ $P = 0.87$	1.68 ± 0.61 $n = 5$ $P = 0.95$	3.22 ± 1.05 $n = 5$ $P = 0.96$	0.19 ± 0.46 $n = 3$ $P = 0.27$	0.59 ± 1.14 $n = 3$ $P = 0.33$	−0.66 ± 0.50 $n = 3$ $P = 0.68$	−0.14 ± 0.56 $n = 3$ $P = 0.14$	0	0.75 ± 0.68 $n = 9$ $P = 0.70$
Ch/Hatsav	2.26 ± 0.57 $n = 11$ $P > 0.99$	1.97 ± 0.79 $n = 6$ $P = 0.95$	0.98 ± 0.43 $n = 10$ $P = 0.95$	1.47 ± 0.61 $n = 11$ $P = 0.97$	0.67 ± 0.28 $n = 7$ $P = 0.95$	1.50 ± 0.41 $n = 8$ $P = 0.99$	−0.18 ± 0.30 $n = 6$ $P = 0.42$	0.10 ± 0.72 $n = 8$ $P = 0.08$	1.40 ± 0.52 $n = 8$ $P = 0.97$	1.40 ± 0.52 $n = 4$ $P = 0.77$	1.23 ± 0.80 $n = 7$ $P = 0.15$	0.17 ± 0.87 0

Upper figure d in each cell is the mean value (with standard error) of $\log_2$ of the ratio between homologous and heterologous HI titers when a given antiserum was the same for the two viruses compared. n is the number of experiments for each pair of compared viruses. P is Student's coefficient of probability for the difference d found for a given number of experiments n. It is expressed as a percentage of probability of the zero hypothesis i.e. the probability that the difference d itself differs significantly from zero. The reliability of the d values (their difference from zero) was accepted as significant if it was in agreement with Fischer's 2nd criterion of probability, namely 95% probability ($P = 0.95$).
The values of d which differ significantly from zero ($P \geq 0.95$) are bold faced.

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(Table 2) In the case of the Yucaipa–Haneda pair, there was two-sided asymmetric cross reactivity, namely

$$\frac{\text{Yucaipa}}{\text{Haneda}} = \frac{1\ 02 \pm 0\ 21}{2\ 54 \pm 0\ 29}$$

In the other pairs related to these four viruses, there was symmetric cross reactivity, the *d* indication being in the range between 1 and 2 $\log_2$

These four strains also differed in the pattern of their cross reactivity with the Israeli isolates For example, PLOC showed no difference with Ty/Kfar-Hess/10B virus and low one-sided asymmetric cross reactivity with the Ch/Hatsav isolate, Robin showed one sided asymmetric cross reactivity with both the native isolates Yucaipa showed a difference with both the native strains displaying two-sided (Ty/Kfar-Hess/10B) and one-sided (Ch/Hatsav) asymmetric cross reactivity, Haneda showed symmetric (Kfar-Hess/10B) and one-sided asymmetric (Ch/Hatsav) cross reactivity (Table 2)

For the other six PMV-2 reference viruses against which there was no antiserum and, hence, two-sided cross reactivities could not be determined, a comparison could be made only with respect to their HI titers with antisera against the 4 reference viruses (Table 2) According to this criterion, some regularities can be noticed Namely all the viruses show a similar relationship to Robin (line 2), the difference between homologous and heterologous HI titers (*d*) ranging between 1 4 and 3 4 $\log_2$ The same pattern was seen with Haneda with *d* ranging between 1 and 2 5 $\log_2$ (line 4), the strain Indonesia/3650 being the only isolate not differing from the homologous virus At the same time, all the viruses showed antigenic similarity with the PLOC virus, *d* being statistically non-significant (close to zero) Yucaipa virus showed similarity with England Italy Indonesia/3589 and Indonesia/3650 (no significant difference of *d* from zero) while it differed from Bangor and Kenya Both the antisera against the two Israeli viruses showed similarity with the viruses Italy, Indonesia/3589 and Kenya, with the other reference strains there were different combinations Ty/Kfar-Hess/10B did not differ from the reference strains, except, Bangor, Ch/Hatsav showed difference also with England and Indonesia/3650

Thus summarizing the above data, it can be concluded that viruses Robin and Haneda are similar by two-way cross reactivity while the viruses Bangor and Kenya, on one hand, and Italy and Indonesia/3589 on the other hand, are similar according to the less strong criterion of the spectrum of antigenic relationships (one-sided cross reactivity) with the four viruses (Yucaipa Robin PLOC and Haneda) Bangor and Kenya by the same criterion are similar to the PLOC virus and show significant difference from Yucaipa Robin and Haneda

HI tests with Israeli PMV-2 isolates

The results of HI tests with the field isolates are shown, according to the year of isolation during 1979–81, in Tables 4–6

In general, there was a considerable heterogeneity among the field strains However certain regularities can be noticed

With Yucaipa antiserum only 6 of 33 strains showed low, although significant, difference $d > 0$ from the homologous titers, of these 5 strains showed differences $d > 1$ Mal/Ramon Ty/Kfar-Hess/10B Ty/Burgata (Table 3), Ty/Nahla/687 (Table 4), and Ch/Petahia (Table 5) For the rest of the strains *d* fluctuated around zero

62

Table 3 Cross HI test with Israeli strains isolated in 197

Antisera	Viruses									
	Mal/ Ramon	Ty/ Kfar Hess/7	Ty/ Kfar Hess/10A	Ty/ Kfar Hess/10B	Ty/ Burgata	Ty/ Beit Shearim 170	Ty/ Sde Yoav 15/T	Ty/ Sde Yoav 15/C	Cegret/ Ramat Gan	M ± SD
Yucaipa	1 34 ± 0 35 $n = 4$ $P = 0 97$	1 42 ± 0 24 $n = 3$ $P = 0 97$	−0 14 ± 0 41 $n = 3$ $P = 0 21$	1 12 ± 0 32 $n = 5$ $P = 0 98$	0 86 ± 0 25 $n = 4$ $P = 0 96$	0 28 ± 0 19 $n = 3$ $P = 0 73$	0 19 ± 0 30 $n = 8$ $P = 0 43$	0 04 ± 1 10 $n = 4$ $P \leqslant 0 06$	−0 28 ± 0 27 $n = 3$ $P = 0 58$	0 54 ± 0 22
Robin	2 60 ± 0 64 $n = 4$ $P = 0 97$	2 13 ± 0 40 $n = 4$ $P = 0 95$	1 01 ± 0 53 $n = 3$ $P = 0 80$	2 04 ± 0 53 $n = 4$ $P = 0 97$	2 40 ± 0 52 $n = 4$ $P = 0 98$	2 00 ± 0 85 $n = 4$ $P = 0 99$	1 51 ± 0 75 $n = 8$ $P = 0 99$	1 90 ± 0 57 $n = 4$ $P = 0 86$	ND	1 95 ± 0 18
PLOC	1 08 ± 0 63 $n = 3$ $P = 0 77$	1 06 ± 0 16 $n = 3$ $P = 0 99$	−0 71 ± 0 70 $n = 4$ $P = 0 61$	1 04 ± 0 58 $n = 3$ $P = 0 79$	1 22 ± 0 67 $n = 4$ $P = 0 84$	ND	−0 20 ± 1 36 $n = 6$ $P = 0 08$	0 40 ± 1 28 $n = 4$ $P = 0 22$	0 60 ± 0 33 $n = 3$ $P = 0 79$	0 56 ± 0 25
Haneda	1 61 ± 0 77 $n = 4$ $P > 0 87$	3 02 ± 0 02 $n = 2$ $P > 0 96$	0 83 ± 0 34 $n = 4$ $P = 0 90$	2 15 ± 0 19 $n = 6$ $P > 0 99$	1 92 ± 0 60 $n = 4$ $P = 0 95$	1 69 ± 0 46 $n = 4$ $P = 0 96$	0 99 ± 0 60 $n = 8$ $P = 0 85$	1 46 ± 0 26 $n = 4$ $P = 0 99$	0 08 ± 0 08 $n = 3$ $P = 0 50$	1 53 ± 0 28
Ty/Kfar Hess 10B	−0 13 ± 0 45 $n = 3$ $P = 0 21$	0 46 ± 0 37 $n = 3$ $P = 0 65$	0 18 ± 0 40 $n = 3$ $P = 0 33$	0	−0 72 ± 0 71 $n = 4$ $P = 0 61$	0 26 ± 0 13 $n = 3$ $P = 0 82$	−0 23 ± 0 50 $n = 10$ $P = 0 37$	0 29 ± 1 12 $n = 8$ $P = 0 23$	ND	0 02 ± 0 15
4Ch/Hatsav	0 77 ± 0 66 $n = 10$ $P = 0 71$	1 40 ± 0 98 $n = 3$ $P = 0 70$	−1 01 ± 0 34 $n = 5$ $P = 0 96$	0 17 ± 0 87 $n = 7$ $P = 0 15$	0 27 ± 0 73 $n = 7$ $P = 0 30$	ND	−0 47 ± 0 64 $n = 8$ $P = 0 49$	−0 44 ± 1 52 $n = 8$ $P = 0 23$	0 27 ± 0 57 $n = 3$ $P = 0 33$	0 12 ± 0 27

The designations are as for Table 2
M ± SD—average d with standard deviation

Table 4 Cross HI test with

Antisera	Viruses						
	Ty/ Beit Herut	Ch/ Mishmeret 589	Ty/ Neot Mordahai	Ty/ Beit Shearim 595	Ty/ Rosh Tsurim	Ty/ Bear Tuvia 676	Ty/ Kfar Harutz
Yucaipa	−0 52 ± 0 66 $n = 3$ $P = 0\ 49$	0 57 ± 0 35 $n = 4$ $P = 0\ 79$	0 60 ± 0 50 $n = 3$ $P = 0\ 65$	0 17 ± 0 38 $n = 3$ $P = 0\ 27$	0 66 ± 0 93 $n = 3$ $P = 0\ 44$	−0 22 ± 0 32 $n = 3$ $P = 0\ 44$	0 63 ± 0 39 $n = 6$ $P = 0\ 83$
Robin	0 62 ± 0 73 $n = 3$ $P = 0\ 85$	2 42 ± 0 78 $n = 4$ $P = 0\ 95$	1 22 ± 0 29 $n = 3$ $P = 0\ 95$	1 80 ± 0 35 $n = 3$ $P = 0\ 96$	1.55 ± 0 50 $n = 4$ $P = 0\ 95$	0 43 ± 0 27 $n = 3$ $P = 0\ 75$	1 92 ± 0 48 $n = 4$ $P = 0\ 96$
PLOC	−0 18 ± 0 55 $n = 3$ $P = 0\ 21$	1 13 ± 0 51 $n = 4$ $P = 0\ 89$	0 20 ± 0 29 $n = 3$ $P = 0\ 44$	1.33 ± 0 39 $n = 4$ $P = 0\ 96$	0 68 ± 0 84 $n = 3$ $P = 0\ 49$	−0 23 ± 0 17 $n = 3$ $P = 0\ 70$	0 52 ± 0 60 $n = 4$ $P = 0\ 54$
Haneda	0 40 ± 0 70 $n = 3$ $P = 0\ 39$	1.80 ± 0 23 $n = 7$ $P > 0\ 99$	1 01 ± 0 23 $n = 6$ $P = 0\ 99$	1 77 ± 0 29 $n = 3$ $P = 0\ 99$	1 84 ± 0 34 $n = 4$ $P = 0\ 99$	0 08 ± 0 48 $n = 3$ $P = 0\ 14$	1 80 ± 0 26 $n = 6$ $P > 0\ 99$
Ty/Kfar Hess 10B	−1 47 ± 1 00 $n = 3$ $P = 0\ 73$	0 97 ± 1 08 $n = 7$ $P = 0\ 61$	0 14 ± 0 71 $n = 4$ $P = 0\ 15$	0 65 ± 1 05 $n = 6$ $P = 0\ 42$	0 00 ± 1 00 $n = 3$	−1 90 ± 0 78 $n = 3$ $P = 0\ 87$	0 07 ± 0 92 $n = 8$ $P = 0\ 08$
Ch/Hatsav	−0 40 ± 0 68 $n = 3$ $P = 0\ 39$	0 21 ± 0 80 $n = 8$ $P = 0\ 23$	0 21 ± 1 18 $n = 7$ $P = 0\ 15$	−0 59 ± 0 38 $n = 8$ $P = 0\ 85$	−0 65 ± 1 12 $n = 3$ $P = 0\ 42$	−1 59 ± 0 95 $n = 5$ $P = 0\ 84$	0 33 ± 1 07 $n = 6$ $P = 0\ 22$

For Robin and Haneda antisera, the values of d demonstrated a generally higher difference In the case of anti-Robin antiserum 12 isolates showed high difference ($2\ 0 \leqslant d \leqslant 3\ 0$), the Ch Nahla strain having shown $d = 3\ 37 \pm 1\ 08$, 11 isolates showed a moderate difference ($1\ 0 \leqslant d \leqslant 2\ 0$) and the rest (10 isolates) showed little or no difference In the case of the anti-Haneda antiserum, 5 isolates showed high difference two of them having shown $d > 3\ 0$, 16 isolates showed moderate difference and the rest (10 isolates) little or no difference

In the case of the anti-PLOC antiserum, 10 isolates showed a moderate difference one isolate showed $d = 3\ 21 \pm 0\ 32$ and the rest (22 isolates) little or no difference

This evident heterogeneity amongst the Israeli strains revealed by the cross reactivity with reference antisera was in contrast to the results manifested by HI tests with 2 antisera against 2 selected Israeli viruses, in which case the values of d showed no significant difference from zero The only exceptions were Ch/Petahia and Ty/Ein-Tsurim strains which showed significant difference from the homologous viruses ($d > 1$) with both the antisera and Coot/Beit-Alfa and Ty/Kfar Hess/7 strains which showed significant difference with one of the antisera

In some cases the d indication had negative (statistically significant) values which meant that the heterologous HI titer was higher than the homologous As such a phenomenon was observed usually when all, or most, of the 6 reference antisera reacted with any virus this might reflect a particular sensitivity of the viral antigen to antibodies, which has been known as the phenomenon of avidity The isolates Ty/Nahla/812 and Ty/Sde-Moshe (Table 4) are the best expressions of such avidity

Elution properties

During the preliminary studies it was noticed that several isolates showed rapid elution after HI tests had been done More systematic evaluation of this phenomenon revealed considerable variation among the strains and isolates tested (Table 6) Of the reference

ted in T980

	Viruses						
Ch/ sav	Ty/ Bear Tuvia 752	Ch/ Mishmeret 803	Ty/ Shalva	Ty/ Nahla 812	Ty/ Sde Moshe	Coot Shluhot	M ± SD
5 ± 0 23 n = 6 P = 0 90	−0 06 ± 0 54 n = 4 P = 0 54	0 06 ± 0 39 n = 3 P = 0 21	−0 19 ± 0 42 n = 5 P = 0 36	−0 26 ± 0 24 n = 3 P = 0 61	0 10 ± 0 27 n = 3 P = 0 27	0 09 ± 0 36 n = 4 P = 0 22	0 23 ± 0 12
.03 ± 0 57 n = 3 = 0 96	1 11 ± 0 26 n = 3 P = 0 95	0 56 ± 1 28 n = 3 P = 0 44	1 36 ± 1 08 n = 4 P = 0 70	0 65 ± 0 55 n = 3 P = 0 65	0 29 ± 0 14 n = 3 P = 0 83	1.30 ± 0 31 n = 3 P = 0 95	1 38 ± 0 22
19 ± 0 50 n = 6 P = 0.29	0 98 ± 0 86 n = 3 P = 0 61	0 71 ± 0 78 n = 3 P = 0 54	−0 69 ± 0 26 n = 3 P = 0 89	−0 31 ± 0 22 n = 3 P = 0 70	−0 26 ± 0 15 n = 3 P = 0 77	0 18 ± 0 26 n = 3 P = 0 44	0 36 ± 0 16
1.50 ± 0.24 n = 8 P ≥ 0 99	0 94 ± 0 31 n = 6 P = 0 97	1.52 ± 0 36 n = 4 P = 0 97	1 22 ± 0 41 n = 6 P = 0 97	−0 56 ± 0 75 n = 3 P = 0 44	0 08 ± 0 58 n = 3 P = 0 07	1 15 ± 0 34 n = 4 P = 0 96	1 17 ± 0 23
0.75 ± 0 68 n = 9 P = 0 70	−0 96 ± 0 58 n = 5 P = 0 84	−0 60 ± 0 72 n = 4 P = 0 52	−1 55 ± 0 66 n = 7 P = 0 94	−0 50 ± 0 69 n = 5 P = 0 29	−0 96 ± 1 00 n = 3 P = 0 58	0 06 ± 0 55 n = 5 P = 0 07	−0 38 ± 0 22
0	−0 08 ± 0 51 n = 9 P = 0 15	−0 13 ± 0 62 n = 8 P = 0 15	−1 06 ± 1 33 n = 6 P = 0 54	−2 32 ± 0 18 n = 4 P > 0 99	−1 67 ± 0 32 n = 5 P > 0 99	−0 88 ± 0 46 n = 7 P = 0 89	−0 54 ± 0 24

sted, only Haneda and Bangor showed full elution which occurred within 4 h ed either no elution after 24 h or only partial elution at this time Thirty-three tes were also tested and these showed considerable heterogeneity in terms of Table 6) Most of the strains (23) were partial or moderate eluters but 5 isolates -eluters and 5 classed as fast eluters

al polypeptides

d preparations of 16 of the Israeli isolates were subjected to SDS–PAGE in on with the Yucaipa and NDV/Ulster viruses [Fig 1 (1) (2) (3)] The overall ptide patterns of the isolates were similar to each other consisting of the same r and approximate size of polypeptides (the lines between the L and HN proteins l(1), are probably impurities) but differed from the reference Yucaipa virus in ion rate of most the polypeptides However more detailed examination revealed ifferences amongst the isolates which were expressed in the position of the F_0 and proteins and the band density of the HN protein By these indications, all the studied s can be distributed into 2 groups Group A included viruses whose F_0 glycoprotein ed faster, the M protein migrated slower and the density of the HN protein band nificantly lower as compared to the respective values of the viruses belonging to B [Fig 1(1) (2) (3)] The difference in the HN band density is most clearly trated in the case of Ty/Neot Mordehai [Fig 1(1)] Ty/Beit-Sheraim [Fig 1(2)] Mal/Ramon [Fig 1(3)] viruses where the above low density of the HN protein is ined with the higher density of the NP and M bands (higher if compared with the ctive bands of the viruses belonging to group B) Group A included the viruses far-Hess/10A, Ty/Kfar-Harutz Tv/Neot-Mordehai Coot/Shluhot [Fig 1(1)], e-Yoav/15/T, Ty/Beit-Shearim/170 Ty/Bear-Tuvia/752 [Fig 1(2)], Mal/Ramon and mmeret [Fig 1(3)] Group B included the viruses Ty/Shalva, C egret/Ramat-Gan,

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Table 5 Cross HI test with Israeli strains isolated in 1981

Antisera	Viruses									
	Ch/ Petahia	Ty/ Lin Tsurim	Ty/ Tsora	Ty/ Talmi Yiel	Ty/ Beit Yitzhak	Ty/ Hatsav T4	Coot/ Beit Alfa	Ty/ Hatsav T6	Ty/ Hatsav T8	M ± SD
Yucaipa	1 17 ± 0 38 n = 4 P = 0 95	ND	−0 16 ± 0 50 n = 4 P = 0 22	−0 40 ± 0 32 n = 3 P = 0 65	−0 77 ± 0 71 n = 3 P = 0 61	−0 23 ± 0 20 n = 3 P = 0 77	0 43 ± 0 41 n = 6 P = 0 64	0 10 ± 0 32 n = 6 P = 0 22	0 27 ± 0 12 n = 5 P = 0 91	0 05 ± 0 21
Robin	2 63 ± 0 54 n = 3 P = 0 96	2 96 ± 0 70 n = 3 P = 0 95	2 19 ± 0 71 n = 4 P = 0 95	0 18 ± 0 11 n = 3 P = 0 75	−0 18 ± 0 20 n = 3 P = 0 54	1 19 ± 0 27 n = 3 P = 0 95	2 00 ± 0 60 n = 4 P = 0 95	1 33 ± 0 26 n = 6 P > 0 99	2 55 ± 0 57 n = 3 P = 0 95	1 65 ± 0 55
PLOC	1 89 ± 0 67 n = 3 P = 0 89	3 21 ± 0 54 n = 3 P > 0 96	1 34 ± 0 67 n = 4 P = 0 88	−0 06 ± 0 23 n = 3 P = 0 21	0 29 ± 0 60 n = 3 P = 0 48	0 36 ± 0 52 n = 3 P = 0 44	0 31 ± 0 29 n = 3 P = 0 61	−0 03 ± 0 42 n = 6 P = 0 08	0 00 ± 0 30 n = 4	0 73 ± 0 42
Haneda	3 01 ± 0 19 n = 3 P > 0 96	2 78 ± 0 66 n = 3 P = 0 95	1 88 ± 0 59 n = 4 P = 0 95	0 93 ± 0 13 n = 3 P > 0 96	0 32 ± 0 66 n = 3 P = 0 33	1 57 ± 0 59 n = 5 P = 0 95	1 81 ± 0 20 n = 6 P > 0 99	1 41 ± 0 44 n = 6 P = 0 98	0 88 ± 0 23 n = 7 P = 0 99	1 62 ± 0 29
Ty/Kfar Hess 10B	1 65 ± 0 65 n = 6 P = 0 95	3 40 ± 0 61 n = 3 P = 0 96	0 21 ± 1 47 n = 5 P = 0 07	−0 21 ± 0 97 n = 5 P = 0 15	−2 31 ± 1 61 n = 3 P = 0 70	1 18 ± 0 84 n = 3 P = 0 70	1 43 ± 0 62 n = 9 P = 0 95	1 61 ± 1 50 n = 6 P = 0 68	−0 27 ± 0 78 n = 3 P = 0 21	0 74 ± 0 54
Ch/Hatsav	1 45 ± 0 54 n = 5 P = 0 95	1 02 ± 0 30 n = 7 P = 0 99	0 14 ± 0 50 n = 7 P = 0 23	−0 94 ± 1 06 n = 5 P = 0 58	0 42 ± 2 20 n = 5 P = 0 15	0 17 ± 0 52 n = 4 P = 0 22	0 71 ± 0 73 n = 8 P = 0 65	0 06 ± 0 34 n = 12 P = 0 15	0 17 ± 0 14 n = 10 P = 0 74	0 36 ± 0 23

The designations are as for Table 3

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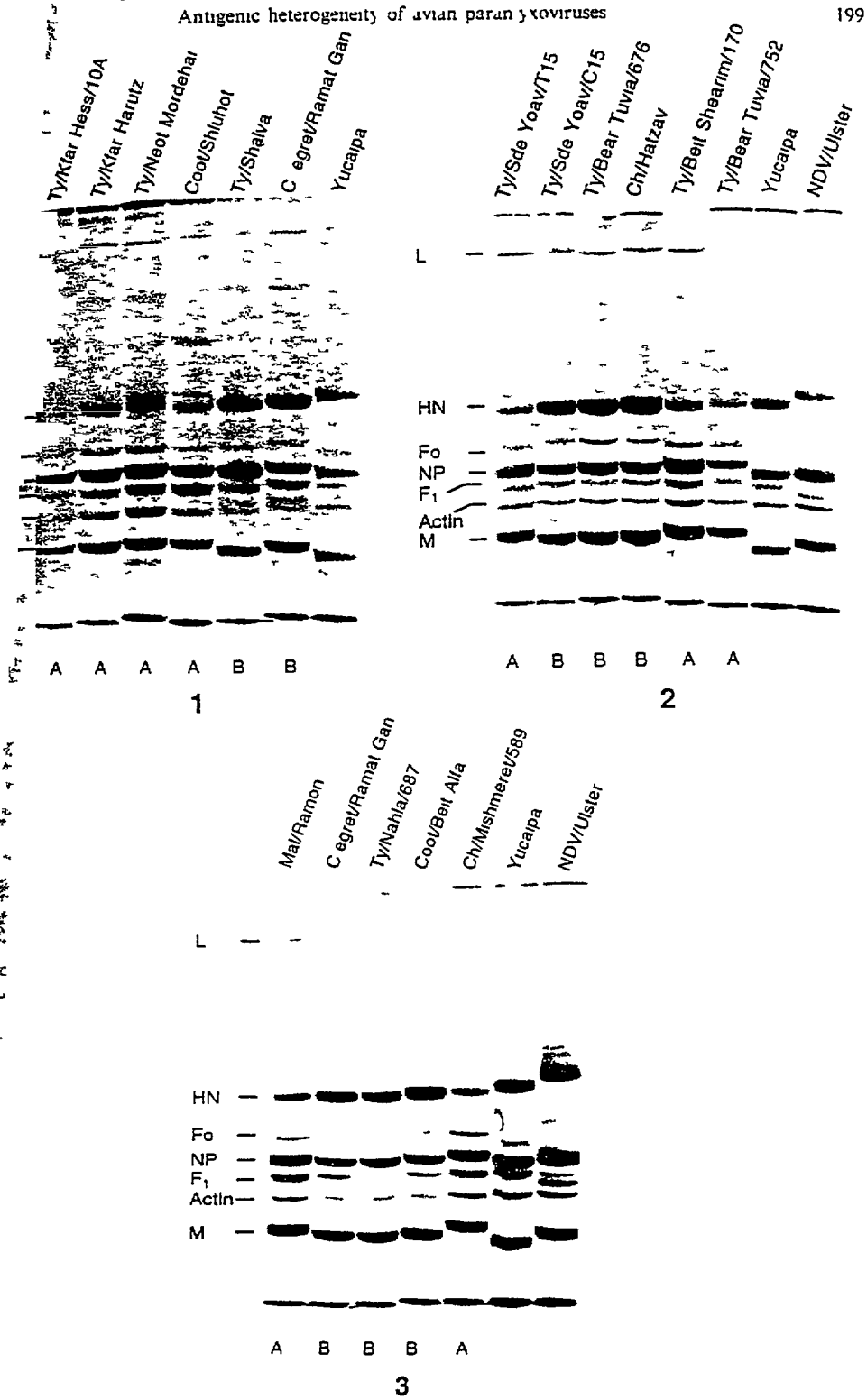

Fig 1 Structural polypeptides of the PMV 2 virus isolates 1 2 3—parts of the figure representing different experiments L HN NP F_1 and M—structural viral polypeptides Act—actin A and B are designations of the two groups into which the Israeli isolates could be distributed

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67

Table 5 Hemagglutination–elution pattern of PMV 2 viruses

Viruses	Non eluters	Partial eluters	Moderate eluters	Fast eluters
Reference	Yucaipa PLOC Italy	Robin England Indonesia/3589 Indonesia/3630 Kenya		Haneda Bangor
Israeli	Ty/Kfar Hess/7 Ty/Kfar Hess/103 Ty/Burgata Ty/Sde Yoav/15/T Ty/Nahla/687	Ty/Beit Shearim/170 Ty/Sde Yoav/15/C Ty/Kfar Harutz Ty/Bear Tuvia/676 Ty/Ein Tsurim Ty/Tsora Ty/Talmi Ehiel Ty Beit Yitzhak Ty/Rosh Tsurim Ty/Hatsav/6/T Ty/Hatsav/8/T	Mal/Ramon Ty/Sde Moshe Ty/Shalva Ch/Hatsav Coot/Beit Alfa Ty/Kfar Hess/10A Coot/Shluhot Ch/Mishmeret/803 Ty/Bear Tuvia/752 Ty Neo Mordehai Ty/Hatsav/4/T Ty/Beit Shearim/595	C egret/Ramat Gan Ty/Beit Herut Ch/Mishmeret/589 Ty/Nahla/812 Ch/Petahia

Explanations are in the text

[Fig 1(1)], Ty/Sde-Yoav/C/15 Ty/Bear-Tuvia/676, Ch/Hatsav [Fig 1(2)] Ty/Nahla/687 and Coot/Beit-Alfa [Fig 1(3)]

DISCUSSION

PMV-2 viruses were isolated from various avian species including domestic and wild birds [2 3 4, 12 13 14, 20, 9, 32 28 15] Antigenic differences between different isolates were clearly noticeable at the very beginning, so that the description Yucaipa-*like* viruses was used [30 29, 1]

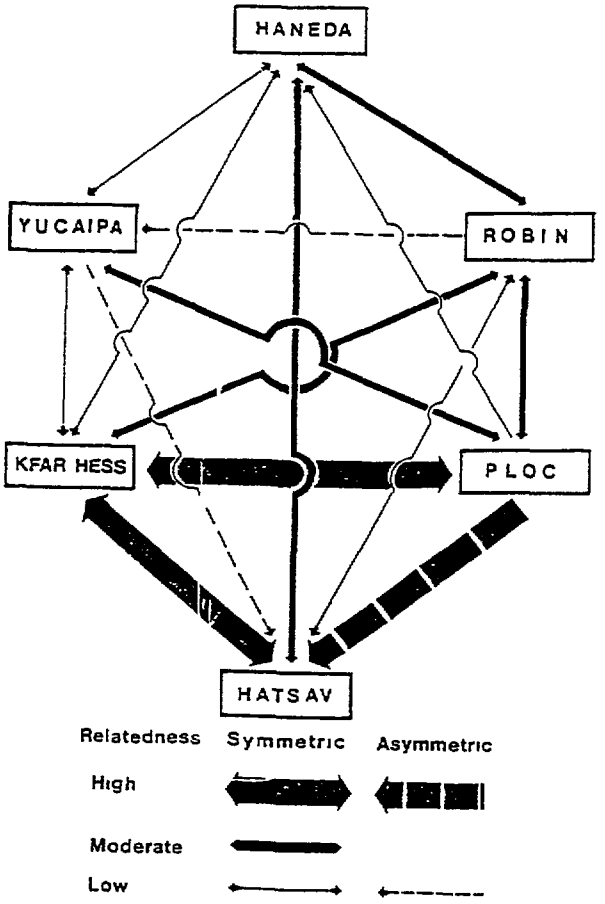
The library of the Israeli PMV-2 isolates used in the present studies is a unique one from several points of view Most of the isolates were obtained during the epizooty of the so-called "turkey disease which spread through all the poultry farms in the country This epizooty was under the continuous observation of the Department of Avian Disease of the Kimron Veterinary Institute and was accompanied by a wide surveillance of feral birds This resulted in accumulation of more than 70 PMV-2 isolates from various hosts—perhaps the largest collection for a single country in the world Such an ecological experiment with continuous surveillance and virus isolation permitted observation of the general dynamics of the 3-year-long epizooty and enabled potential assessment of possible antigenic variations

The antigenic variations detected however were difficult to quantitate because of the absence of steady reference background Indeed the reference PMV-2 viruses used in the studies showed multiple antigenic variations (Table 2) However certain regularities can be noticed even on such unsteady ground Namely in general the PLOC virus showed lesser differences from the other viruses when anti-PLOC antiserum was used the difference by *d* indication was significant only with 2 viruses (Yucaipa and Bangor) from the 12 viruses used in the studies (Table 2) These antigenic differences were however one-sided asymmetric Besides, the PLOC virus showed no significant difference in the cross reactivity with the Israeli Ty/Kfar-Hess/10B virus and its difference from the other Israeli isolate—Ch/Hatsav—was minimal (less than 1 $\log_2$) and one-sided asymmetric (Table 2) In contrast, Robin and Haneda viruses demonstrated marked variability

68

'n showed a significant difference between Robin and the other viruses -s (Table 2) Similar results were obtained with anti-Haneda serum with of Indonesia/3650 virus, in most cases, the cross reactivity was sym- virus showed, in most cases, asymmetric cross reactivity and large uters from the viruses whose antisera were used in the studies Of the -s whose antisera were not used in the studies, the most prominent was showing difference by *d* values with antisera against all the viruses used in Table 2)

enic interrelationships, which have been displayed graphically (Fig 2), dness, almost identity (in the limits of serological criteria), between the es and the PLOC virus In general, the PLOC virus has the closest related- her members of the graph from 5 possible interconnections, 2 designate ess, 2—moderate, and 1—low The Robin virus has moderate cross reaction and low with 2 others Haneda virus displays 2 moderate and 3 low relations



Graphic display of antigenic re ationships between prototype and Israeli reference PMV-2 viruses

and Yucaipa virus shows only 1 moderate (with PLOC) and 4 low relationships This picture is compatible with the theories of the 'common and "serotype specific' portions on the genomic and, correspondingly, the antigenic level [21] and the notions of "conserved ' and "variable" regions [31] and 'strain-specific ' epitope on the NDV HN molecule recognized by monoclonal antibodies In accordance with this, one can speculate that amongst the PMV-2 viruses used in the studies the PLOC virus has the largest "common" portion while the Yucaipa virus has the smallest

The similarity of the Israeli isolates to the PLOC virus is supported by data on the PLOC's relationships with all the other Israeli isolates (Tables 3–5) From the total of 33 Israeli isolates, a significant difference by *d* indication was shown in only 2 cases with Ty/Beit-Shearim/595 (Table 5) and Ty/Ein-Tsurim (Table 6)

On the basis of the above data it can be suggested that the PLOC virus is the ancestor of the Israeli isolates or, that both have a common ancestor, from whom they have not drifted far away in a fan-like direction From the ecological point of view, the relationship of the Israeli isolates with PLOC virus would be expected since Israel is on the migratory route of passerines travelling from Europe to West Africa

However, the main objective of the studies was an attempt to find out if antigenic drift could occur during the epizooty and could be revealed by analyzing antigenic heterogeneity among the consecutively isolated viruses The heterogeneity was demonstrated by different parameters

Firstly, detailed comparison of 2 Israeli isolates which were selected as "native reference' viruses—Ty/Kfar-Hess/10B and Ch/Hatsav—with the reference PMV-2 strains revealed differences between them although direct comparison of both did not show significant difference in cross reactivity (Table 2)

$$\frac{\text{Ty/Kfar-Hess/10B}}{\text{Ch/Hatsav}} = \frac{0\ 75 \pm 0\ 68}{0\ 17 \pm 0\ 87}$$

However, the 2 viruses clearly differed from each other by their cross reactivity with the other PMV-2 Namely, Ty/Kfar-Hess/10B showed two-sided cross reactivity while Ch/Hatsav showed one-sided asymmetric cross reactivity with Yucaipa virus Besides, there was no parallelism between the 2 viruses in their cross reactivity with the PLOC, Haneda, and Indonesia/3650 viruses Such a phenomenon, when there is no significant antigenic difference between 2 viruses, but they differ by the spectrum of their cross reactivity with some other viruses has been described earlier [21, 22]

Secondly, there were several isolates which showed various differences from the native reference viruses, namely, Ch/Petahia, Ty/Ein-Tsurim, Coot/Beit-Alfa (Table 5) In addition, there were various differences between the isolates in their pattern of relationships by *d* indication with the 4 reference PMV-2 viruses whose antisera were used in the studies On the basis of these patterns, it was possible to distribute the majority of the isolates into 4 groups

Group 1

The pattern of the relationships—significant differences between the isolates and the Robin and Haneda viruses, but no difference with Yucaipa, PLOC and the "native reference" viruses This is the most representative group containing the following 10 isolates Ty/Beit-Shearim/170 (Table 3), Ch/Mishmeret/589, Ty/Neot-Mordehai,

70

r-Harutz, Ch/Hatsav, Ty/Bear-Tuvia/752 (Table 4), Ty/Tsora, Ty/Hatsav/4/T, -av/6/T and Ty/Hatsav/8/T (Table 5)

2

ttern of the relationships—no difference between the isolates and any of the 6 ce (including "native reference") viruses The following 6 viruses fall into this Ty/Kfar-Hess/10A (Table 3), Ty/Beit-Herut, Ty/Bear-Tuvia/676, Ty/Nahla/812, Moshe (Table 4) and Ty/Beit-Yitzhak (Table 5)

3

pattern of the relationships—differences between the isolates and Haneda e virus with no difference concerning the other 5 reference viruses The following ll into this group Ty/Sde-Yoav/15/C, Ty/Sde-Yoav/15/T (Table 3), Ty/Rosh- Ch/Mishmeret/803, Ty/Shalva, Coot/Shluhot (Table 4) and Ty/Talmi-Ehiel 5)

ttern of the relationships—significant differences between the isolates and Robin and Haneda viruses with no difference from the other reference viruses isolates fall into this group Ty/Kfar-Hess/7, Ty/Kfar-Hess/10B, Ty/Burgata), and Ty/Nahla/687 (Table 4)

tes which did not fall into these groups are heterogeneous in various ns of the relationship patterns It is possible, for example to form another cluding 3 isolates having shown differences namely with the native refer es Ch/Petahia, Ty/Ein-Tsurim and Coot/Beit-Alfa However, the latter iffer from each other by their relationships pattern with the other reference

s the antigenic differences some of the strains showed significantly high values of the *d* indication [(Ty/Nahla/812, Ty/Sde-Moshe, Ty/Bear-Tuvia/676 , Ty/Kfar-Hess/10A (Table 3)] We consider this fact as an expression of [18]

e results have demonstrated wide and multiple antigenic heterogeneity amongst s with no clear picture of regular antigenic evolution (drift) However certain n the heterogeneity pattern expressed in a relative prevalence of the above- d groups of antigenic variants during the 3-year-period of observation can be mely, 3 out of the 4 viruses of group 4 were isolated during the first quarter en only 1 such virus was isolated in 1980, and nothing in 1981 At the same 1 virus from the total of 10 belonging to group 1 was isolated in 1979 while es were isolated in 1980 and 1981, respectively From the viruses belonging only 1 was isolated in 1979 and 4 and 1 were then isola ed in 1980 and 1981 y, and from the viruses belonging to group 3, 1 was isolated in 1979 and 4 and n isolated in 1980 and 1981, respectively Thus, the viruses of group 4 evidently arlier in the epizooty while the isolation of the viruses of group 1 was distributed 0–81

among the viruses isolated in 1979, there were already representatives of all apart from some other variants which are not included in those groups It

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71

has not been possible to arrange the isolates in an order displaying consecutive accumulation of changes along with a time axis which could demonstrate an antigenic drift According to the dynamics of isolations of different antigenic variants, it seems that the isolates did not derive from a certain ancestral strain(s) as a result of consecutive changes but that there was a co-circulation of a number of PMV-2 variants which appeared at the very beginning of the epizooty

This suggestion is supported by the results on the hemagglutination–elution pattern (Table 6) On the one hand, there is a certain connection between antigenicity and the hemagglutination–elution pattern, namely, 4 from the 5 non-eluter viruses are those belonging to group 4 from which 3 were isolated in 1979 But this is the only clear correlation on the background of flat randomness However, to some extent a chronological relationship can be seen in the groupings of strains on elution ability in that the earlier isolates were non-eluters and fast eluters appeared in 1980 (Table 6) However some strains isolated in 1980, and even in 1981, were moderate eluters Moreover it was noticed that during passaging, the elution properties of certain strains were changing On the basis of these data, we suggest that within the population of the PMV-2 viruses there should be two sorts of virions in respect to their elution–hemagglutination property— 'non-eluter and "fast eluter particles In accordance with this the entire spectrum of differences found should be due to different proportions of non-eluter and fast eluter particles in the heterogeneous viral populations of the corresponding strains This is one more indication that during the studied period (1979–1981) in the avian population in Israel there was co-circulation of different variants differing, at least in antigenicity and elution–hemagglutination properties

The distribution of the isolates by the PAGE pattern of the viral protein migration rate has also revealed no correlation with the previously described grouping of the isolates by both antigenicity and elution-hemagglutination pattern Namely, if comparing the grouping by PAGE pattern with that by antigenicity it can be seen that both groups A and B (PAGE pattern) include viruses belonging to different groups qualified by antigenicity and that some viruses belonging to the same groups, qualified by antigenicity, may belong to different groups qualified by PAGE The same situation can be seen if comparing the grouping bv PAGE with that by elution–hemagglutination pattern Namelv most of the viruses (5 out of 9) belonging to group A are moderate eluters (Ty/Kfar/Hess/10A Ty/Neot-Mordehai, Coot/Shluhot Ty/Bear-Tuvia/752 and Mal/Ramon) but 2 viruses are partial eluters (Ty/Kfar-Harutz and Ty/Beit-Shearim), 1 is a non-eluter (Ty/Sde Yoav/15/T) and 1 is a fast-eluter (Ch/Mishmeret/589) group B includes Ty/Nahla/687 which is a non-eluter on the one hand, and C egret/Ramat-Gan which is a fast eluter on the other hand [Table 6 and Fig 1 (1) (2) (3)]

Thus, the general conclusion from the studies is that the PMV-2 viruses isolated in Israel during the period 1979–81 demonstrate heterogeneity by antigenicity of the HN glycoprotein, elution–hemagglutination pattern and PAGE protein migration pattern with no evident correlation between the three indications, i e grouping of the isolates, by any of the above criteria, does not coincide with each other Together with this there is no evident correlation between the above parameters and the chronology of the virus isolations This suggests that during the epizooty there was a co-circulation of different variants of the PMV-2 virus, varying by at least three different properties, which were not necessarily combined The most striking fact in this respect concerns the viruses Ty/Sde-Yoav/15/T and Ty/Sde-Yoav/15/C (Table 1) which were isolated from tracheal and cloacal swabs

72

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respectively, taken from the same individual turkey and which differed by antigenicity (Table 3), elution–hemagglutination (Table 6) and PAGE protein migration (Fig 1) patterns In addition to these facts, there have been some observations on changing elution–hemagglutination pattern together with consecutive virus passaging

In connection with this, the intriguing question is whether such wide heterogeneity of the Israeli PMV-2 isolates is a result of remarkable variability of a hypothetical initially homogeneous virus, which would cause the epizooty, and be the ancestor of all the varying isolates, or the causative viral factor was heterogeneous (consisted of heterogeneous virion population) from the very beginning of the epizooty The above-described experimental evidence demonstrating the heterogeneity among the early isolates supports the latter hypothesis This, in turn leads to two alternative suggestions

(1) The PMV-2 virus had been circulating in the local avian reservoir long before the beginning of the described outbreak which means that it was enzootic rather than epizootic Enzootic origin of the local PMV-2 isolations would explain evolutionary antigenic and other biological changes which led to accumulation of the corresponding variants In such a case there should be a certain ' provocative factor which would "wake up the sleeping" enzootic PMV-2 virus Turkey rhinotracheitis (TRT) pneumovirus would have been such a provocative factor—the disease was diagnosed by clinical syndrome in 1978 the viral agents were isolated in 1980 and were identified as TRT in 1987 [41, 42]

(2) The PMV-2 virus was brought into the local avian reservoir from abroad (real epizooty) probably via migrating birds, but the 'imported virus was already heterogeneous

The choice between the two alternatives is due to the existing ecological evidence According to that, dozens of HA agents identified either as influenza viruses or APMVs were isolated before and after the epizooty with no PMV-2 among them, although massive surveillance and swabbing of domestic and feral birds began before the epizooty and went on in the same intensity after it This is not compatible with the first alternative but corresponds to the second one Since the Mal/Ramon virus has been the earliest Israeli PMV-2 isolation preceding the epizooty in poultry, it is tempting to speculate about interspecies transfer of the PMV 2 virus from wild migrating to local domestic birds However in such a case the Mal/Ramon virus was not a pure genetic ancestor but was initially heterogeneous

The hypothesis about the initial heterogeneity is compatible with the views about rapid evolution of RNA viruses [17] and microheterogeneity of RNA genomes [39] based on the quasispecies concept [11] According to these views, the multiple mutational changes are not evolved sequentially but are diverged from a common ancestor and, hence, together with the main gene sequence, there is a "quasispecies mutant distribution [10, 11] These rapidly mutating subpopulations can remain remarkably stable under certain conditions of replication but when the conditions are suddenly changed, the equilibrium between the main virus population and multiple subpopulations becomes disturbed resulting in selection of a certain subpopulation(s) which then become(s) the main one(s) This displays an extremely rapid evolution and an acute outbreak of a virus disease is an effective factor for such rapidity

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The above considerations are relevant, not only to the heterogeneity of the local PMV-2 isolates, but to the reference PMV-2 viruses as well since they have also shown a wide heterogeneity according to the above three indications

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74

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75

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DELINEATION OF ANTIGENIC EPITOPES ON THE HEMAGGLUTININ NEURAMINIDASE AND FUSION GLYCOPROTEINS OF APMV-2/YUCAIPA VIRUS BY MEANS OF MONOCLONAL ANTIBODIES

A Panshin[1], E Shihmanter[1], Y Weisman[1], C Örvell[2] & M Lipkind[1]

1 INTRODUCTION

Yucaipa virus belongs to the avian paramyxovirus serotype 2 (PMV-2) Viral genome of PMV 2 contains 6 genes coding for 3 envelope proteins matrix (M) fusion (F) and hemagglutinin-neuraminidase (HN) and three „core„ proteins nucleoprotein (NP) phosphoprotein (P) and „large, protein (L) Yucaipa virus is characterized by the presence of two types of glycoprotein spikes protruding from the cellularly-derived viral membrane, namely HN and F The HN and F glycoproteins of paramyxoviruses play a key role in the initiation of infection The lager of the two glycoproteins, HN (mol wait 74 kDa), responsible for mediating the attachment of the virus to the host cell receptor The second glycoprotein, F protein (mol wait 52), interacts with cell plasma membrane causing fusion between viral envelope and the plasma membrane, which results in subsequent penetration and internalization of the viral nucleocapsid Several groups have used monoclonal antibodies (MABs) to generate functional profiles of the antigenic cites on the HN and F glycoproteins of paramyxoviruses It was shown that mouse monoclonal antibodies prepared against APMV-1 and APMV-3 have enabled differentiation and grouping of strains and isolates within those serotypes It seemed likely that MABs directed against PMV-2 would be useful in grouping or differentiating between strains of this serogroup In the present studies a panel of 12 anti-HN and 6 anti-F MABs permitted to extend antigenic characterization of the HN and F proteins by detailed epitope mapping

2 MATERIALS AND METHODS

Viruses The PMV-2/chicken/California/Yucaipa (Yuc) strain kindly provided by Dr D J Alexander (Central Veterinary Laboratory, Weybridge, UK) was used for preparation of MABs The PMV-2 strains PMV-2/Ty/Kazakhstan (Kaz), and PMV-2/Broniza (Bron) were obtained from Prof M Sayatov (Institute of Microbiology and Virology - Academy of Sciences of the Republic of Kazakhstan, Almaty, Republic of Kazakhstan) Two reference strains, PMV-2/mannikin/Japan/Haneda/35/76 (Han) and PMV-2/Sturnus contra/Indonesia/3589/79 (Ind), and two local field isolates Ty/Kfar

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Hess/7/T/79 3 and Chicken/Hatzav/734/B/80 11, were obtained from the collection of the Kimron Veterinary Institute (Israel)

Propagation of the viruses was done as described below The viruses were inoculated into the allantoic cavity of the 10-11-days-old chick embryonated eggs which were incubated till the period of 3 days post inoculation The hemagglutinating (HA) agents serologically identified as PMV 2 were undergone a minimal number of passages (usually 1-2) through embryonated eggs in order to accumulate an amount of virus of high titer for a virus stock stored at -70°C The virus purification was carried out by differential centrifugation through sucrose density gradients Protein quantification was done using Coomassie Protein Assay Reagent (PIERCE)

Hybridoma production and MAB screening MABs were prepared by fusing spleen cells from Yuc-immunized BALB/c mice with the mouse myeloma cell line SP2/0 The viral envelope fraction obtained by n-octyl-D-glycoside treatment of the purified virus preparation and containing hemagglutinin-neuraminidase (HN) fusion (F) and the M viral proteins was used for the immunization of mice as described previously (Panshin et al , 1995, 1997) The screening of the MAB-secreting hybridoma clones was performed by indirect ELISA using purified virus (Yuc and Bron) as antigen and hemagglutination inhibition test Selected MAB-secreting hybridoma clones were inoculated into pristane-primed BALB/c mice Ascitic fluids were collected 1-2 weeks later

MAB characterization The MABs were characterized for specificity by radioimmune precipitation assay (RIPA) using labelling of viral proteins with ^{35}S-methionine Western blotting analysis (WB), and ELISA with purified HN from Yuc (HN-ELISA) RIPA and TAS-ELISA were performed as described previously (Panshin et al 1995) IB was carried out as described by Samson (1986)

Competitive binding assay Competitive binding assay was carried out according to Nishikawa et al (1987) with some modifications namely the usage of biotin-labelled (instead of peroxidase-labelled) MABs and correspondingly ExtrAvidin TM-peroxydase (Paton et al , 1991)

Determination of MAB isotype Isotypes of the monoclonal antibodies were determined by Ouchterlony immunodiffusion test using Mouse Monoclonal Typing KIT (The Binding Site, Birmingham, UK)

Serology tests Hemagglutination (HI) test was performed according to the standard procedures

3 RESULTS

Specificity of monoclonal antibodies to the envelope proteins of PMV-2

A total of 18 MABs were screened for specificity by immunoprecipitation of PMV-2/chicken/California/Yucaipa-infected and [^{35}S]methionine-labeled BHK cell lysates As it is presented in Table 1, twelve MABs were found to be specific for HN glycoprotein All 12 MABs precipitated HN protein and 11 from them inhibited viral hemagglutinating activity The immunoprecipitates by six other antibodies contained F glycoprotein To determine additional characteristics of these antibodies, virion proteins were separated by SDS-PAGE, transferred to nitrocellulose membrane, and reacted with MABs All the MABs did not bind the viral proteins at all if Western blotting technique was performed according to the standard procedures, in reducing conditions with boiling

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Table 1 Characterization of 18 MABs against PMV-2/ch/California/Yucaipa

MAB	Class/ Subclass	ELISA Vir[1]	ELISA HN[2]	Western blotting analysis[4]	RIPA	HI Titer
24c9	IgG1	3[3]	3	neg	HN (74 kDa)	1 64
46f3	IgG2A	3	3	neg	HN (74 kDa)	1 512
46c8	IgG1	3	3	neg	HN (74 kDa)	1 32
6e2	IgG1	3	3	neg	HN (74 kDa)	1 256
28g3	IgG1	2	2	neg	HN (74 kDa)	1 256
26d10	IgG1	3	3	neg	HN (74 kDa)	1 2048
22c11	IgG1	3	3	neg	HN (74 kDa)	1 512
7c11	IgG2a	3	3	neg	HN (74 kDa)	1 8192
46b8	IgG1	3	3	neg	HN (74 kDa)	1 512
22g5	IgG1	3	3	neg	HN (74 kDa)	1 64
27e9	IgG1	2	2	neg	HN (74 kDa)	1 256
20f9	IgG1	3*	3*	neg	HN (74 kDa)	<1 8
27g5	IgG1	3	-	neg	F (51- 52 kDa)	<1 8
24c3	IgG2A	3	-	neg	F (51- 52 kDa)	<1 8
2g8	IgG1	3	-	neg	F (51- 52 kDa)	<1 8
46c11	IgG1	3	-	neg	F (51- 52 kDa)	<1 8
28e8	IgG1	3	-	neg	F (51- 52 kDa)	<1 8
25d8	IgG1	3	-	neg	F (51- 52 kDa)	<1 8

Class/Subclass of MABs were determined by Mouse monoclonal typing KIT (RK008 The Binding Site)

[1] ELISA – PMV-2/chicken/California/Yucaipa (Yuc) was used as antigen, [2] ELISA - purified HN from Yuc was used as antigen, * PMV-2/Bron was used as antigen, [3] ELISA - intensity of reaction with MAB diluted 1 1000 (+3) - A_{405}>2 (+2) - 2>A_{405}>1 (+1) - 1>A_{405}>0 5, (-) - A_{405}<0 5x

[4] WB technique was applied to Yuc proteins which were electrophoresed in reducing conditions with prior boiling

Competitive binding assay of anti F MABs

The competitive binding assay (Table 2) revealed three non-overlapping MAB groups (1, 2, 3) and two additional groups (1 2 and 2 3) MAB 2g8 (1 2 group) competes with the MABs belonging to the groups 1 and 2 MAB 28e8 (group 2 3) competes with the MABs belonging to the groups 2 and 3

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Table 2 Epitope assignments of anti-F MABs

Competing Antibodies	Biotinylated Antibody						Antigenic sites
	27g5	24c3	2g8	46c11	28e8	25d8	
27g5	+	+	+	-	-	-	F1
24c3	+	+	+	-	-	-	
2g8	+	+	+	+	+	-	F1 2
46c11	-	-	+	+	+	-	F2
28e8	-	-	-	+	+	+	F2 3
25d8	-	-	-	-	+	+	F3

[+] - >60% competition, [+/-] - 50-60% competition, [-] - <50% competition

Competitive binding assay with anti HN MABs

Antigenic mapping of the HN protein was carried out by reciprocal competitive binding assay As it can see in figure 3 on the basis of the competitive test results all the MABs were distributed into eight groups Amongst them there were five non-overlapping groups (1, 2 3 4 and 5) which included the MABs competing only with MABs belonging to the same group Besides those there were three additional groups namely 1 2 group, containing MAB 46f3 competing with the MABs belonging to the 1 and 2 groups 2 3 group containing MAB 6e2 competing with the MABs belonging to the 2 and 3 groups 6 group containing MABs 20f9 and 27e9 competing with the MABs belonging to the 1 1 2, 2 3 4 and 5 groups Thus at least 8 antigenic sites were detected on HN glycoprotein of Yuc by means of the panel of MABs (Table 3)

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Table 3 Epitope assignments of anti-HN MABs

Competing Antibodies	Biotinylated Antibody												Antigenic sites
	24c9	46f3	46c8	6e2	26d10	28g3	22c11	7c11	22g5	46b8	20f9	27e9	
24c9	+	+	-	-	-	-	-	-	-	-	+	+	HN1
46f3	+	+	+	-	-	-	-	-	-	-	+	+	HN1 2
46c8	-	+	+	+	-	-	-	-	-	-	-	-	HN2
6e2	-	-	+	+	+	+	-	-	-	-	+	+	HN2 3
26d10	-	-	-	+	+	+	-	-	-	-	-	-	HN3
28g3	-	-	-	+	+	+	-	-	-	-	-	-	
22c11	-	-	-	-	-	-	+	+	-	-	+	+	HN4
7c11	-	-	-	-	-	-	+	+	-	-	-	+	
22g5	-	-	-	-	-	-	-	-	+	+	+	+	HN5
46b8	-	-	-	-	-	-	-	-	+	+	-	+	
20f9	+	+	-	+	-	-	+	-	+	-	+	+	HN6 a
27e9	+	+	-	+	-	-	+	+	+	+	+	+	HN6 b

PMV-2 strains characterization

The above MAB panel was used for antigenic characterization by ELISA of 7 APMV-2 strains, including 5 reference strains and 2 field isolates As it can be seen in Tables 4 and 5 five antigenic sites HN protein (site HN1, site HN1 2 site HN3 site HN4 and site HN5) and two sites of F protein (site F1 and site F1 2)- are remarkably conserved Namely, all the MABs specific to these sites reacted with all the studied PMV-2 strains MABs specific to the site HN6 showed diverse pattern On one hand, the epitope HN6a was remarkably conserved like the above conserved epitopes of the sites HN1 HN1 HN1 2, HN3 HN4 and HN5 On the other hand, epitope HN6b showed definite variability the respective MABs showed negative reaction with 3 out of the 7 APMV-2 strains The other two antigeic sites of HN (site HN2 and site HN3) and three antigenic sites of F (site F2 site F3 and site F2 3) displayed intermediate attitude Namely, showed definite variability the respective MABs showed negative reaction with 1-2 out of the 7 viruses

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Table 4 Reactivities of anti-HN monoclonal antibodies with APMV-2 strains

VIRUSES	ANTI-HN MONOCLONL ANTIBODIES											
	24 c9	46 f3	46 c8	6 e2	28 g3	26 d10	22 c11	7 c11	46 b8	22 g5	27 e9	20 f9
PMV-2/Broniza	+	+	+	+	+	+	+	+	+	+	+	+
PMV-2/chicken/California/Yucaipa	+	+	+	+	+	+	+	+	+	+	+	-
PMV-2/Ty/Kazakhstan	+	+	+	+	+	+	+	+	+	+	+	-
PMV-2/mannikin/Japan/Haneda/35/76	+	+	-	+	+	+	+	+	+	+	+	-
PMV-2/Sturnus contra/Indonesia/3589/79	+	+	-	+	+	+	+	+	+	+	+	+
Ty/Kfar Hess/7/T/79 3	+	+	+	-	+	+	+	+	+	+	+	+
Chicken/Hatzav/734/B/80 11	+	+	+	+	+	+	+	+	+	+	+	+
Antigenic sites	HN 1	HN 1 2	HN 2	HN 2 3	HN 3		HN 4		HN 5		HN 6a	HN 6b

Reactivity of each MAB with different APMV-2 strains is expressed as a result of their binding (measured by ELISA) to the respective strains The values represent the average of three separate experiments Ascites fluids were used as the sources of antibodies (+) positive binding (-) negative binding

Table 5 Reactivities of anti-F monoclonal antibodies with APMV-2 strains

VIRUSES	ANTI-F MONOCLONAL ANTIBODIES					
	27g5	24c3	2g8	46c11	28e8	25d8
PMV-2/Broniza	+	+	+	+	+	+
PMV-2/chicken/California/Yucaipa	+	+	+	+	+	+
PMV-2/Ty/Kazakhstan	+	+	+	+	+	+
PMV-2/mannikin/Japan/Haneda/35/76	+	+	+	+	+	+
PMV-2/Sturnus contra/Indonesia/3589/79	+	+	+	+	+	+
Ty/Kfar Hess/7/T/79 3	+	+	+	+	+	+
Chicken/Hatzav/734/B/80 11	+	+	+	-	-	-
Antigenic sites	F1		F1 2	F2	F2 3	F3

Thus by MAB binding pattern 7 PMV-2 strains presented in table 4 and 5 can be divide into six antigenic variants These results are compatible with the data published by Ozdemir et al (1990)

4 DISCUSSION

The panel used in the present studies includes 12 anti-HN and 6 anti-F MABs i e more than the panel described by Ozdemir et al (1990) and Mao et al (1996), which contained 3 and 10 anti-HN MABs respectively By the usage of the avidin-biotin system instead of the commonly used peroxydase technique, a higher sensitivity in the case of the competitive blocking test was achieved This provided a clearer determi-

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nation of the HN and F protein antigenic sites The competitive blocking test revealed five non-overlapping and tree overlapping sites on HN and three non-overlapping and two overlapping sites on F Thus, mapping of the HN and F proteins of PMV-2 virus has shown that these glycoproteins have antigenic structures similar to those at other paramixoviruses (Iorio et al , 1987, Panshin et al , 1997, 1998)

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5 SUMMARY

Viruses of the avian paramyxovirus serotype 2 (APMV-2/Yucaipa) have been shown to be widespread among turkeys Hybridomas secreting monoclonal antibodies (MAB) against the prototype strain Yucaipa were prepared by fusing of spleen cells from the APMV-2/Yucaipa-immunized BALB/c mice with the mouse myeloma cell line SP2/0 The viral envelope fraction obtained by n-octyl-D-glycoside treatment of the purified virus preparation and containing hemagglutinin-neuraminidase (HN) fusion (F) and matrix (M) viral proteins was used for the immunization of mice The hybridomas were screened for MAB production by ELISA using the purified virus as antigen MAB-secreting hybridoma clones were inoculated into pristane-primed BALB/c mice Specificity of the MABs toward HN F and M proteins was proven by conventional radioimmunoprecipitation assay Two MAB panels including MABs against glycoproteins HN (12) and F (6) were obtained

Antigenic epitope mapping of HN and F proteins by competitive antibody-binding assay was performed with these panels using biotin-amidocaproate-N-hydroxy-succinimide ester-labeled MABs At least, five non-overlapping and three overlapping sites on HN and three non-overlapping and two overlapping sites on F proteins have been revealed

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FINAL REPORT

Covering period September 1991 - September 1998

"Antigenic and Functional Properties of Hemagglutinin-Neuraminidase Glycoprotein of Avian Paramyxoviruses"

Appendices

Tables & Figures

85

Table 1 Thermostability of HA and Nase activities of HN glycoprotein of various APMV-2 (Yucaipa-like) viruses

Serotype	H A activity		Nase activity	
	NT	T	NT	T
Yucaipa	64	<2	0 260	0
Finch/Bangor	64	<2	0 3[illegible]	0 074
Robin/Hiddensee	64	<2	0 333	0 060
Weaver/Senegal	64	16	0 391	0 045
Mannikin/Haneda	128	<2	0 315	0 060
Finch/England	64	3[illegible]	0 547	0 392
Gadwall/Kenya	128	<2	0 188	0 040
Starling/Indonesia	64	16	0 512	0 385

Table 2 Activity of anti-mumps MABs against ERV

				ELISA		
clone	mouse	specificity	epitope	mumps	ERV	Sendai
687	1 227	HN	HN 9	+	+	-
741	1 218	HN		+	+	-
1 818	549	HN		+	+	-
1 933	572	HN	HN 1	+	+	-
743	1 173	HN	HN 9	+	+	-
1 992	583	HN	HN 4	+	+	-
2 048	614	HN	HN 9	+	+	-
2 068	621	HN	HN 2	+	+	-
5 374	1 100	HN	HN 5	+	+	-
2 072		HN	HN 7	+	+	-
2 082	629	HN	HN 9	+	+	-
2 100	637	HN		+	+	-
2 034	678	HN	HN 9	+	+	-
2 170	688	HN	HN 3	+	+	-
2 073	624	HN	HN 7	+	+	-
2 075	625	HN	HN 8	+	+	-
5 342	1 096	HN	HN 9	+	+	-
5 500	1 115	HN	HN 6	+	+	-
652	256	NP	NP 1	+	+	-
673	259	NP		+	+	-
696	536	NP	NP 2	+	+	-
705	274	NP	NP 3	+	+	-
2 004	676	NP	NP 3	+	+	-
728	288	NP	NP 4	+	+	-
781	303	NP	NP 5	+	+	-
1 806	546	NP		+	+	-
1 990	582	NP		+	+	-
2 002	588	NP	NP 6	+	+	-
2 045	610	NP	NP 7	+	+	-
2 050	616	NP	NP 8	+	+	-
2 099	636	NP	NP 9	+	+	-
2 132	650	NP	NP 10	+	+	-
2 142	655	NP	NP 11	+	+	-
2 054	661	NP	NP 12	+	+	-
1 999	697	NP	NP 13	+	+	-
632	273	P	P 1	+	+	-
680	269	P	P 2	+	+	-

Table 2 continuation

clone	mouse	specificity	epitope	ELISA mumps	ERV	Sendai
2 005	589	P	P 3	+	+	-
2 037	604	P	P 4	+	+	-
689	1 200	P	P 5	+	+	-
2 060	617	P	P 6	+	+	-
703	1 247	P	P 7	+	+	-
2 067	620	P	P 8	+	+	-
2 069	622	P	P 9	+	+	-
1 997	675	P	P 10	+	+	-
654	257	M	M 1	+	+	-
657	249	M	M 2	+	+	-
693	263	M	M 3	+	+	-
745	291	M	M 4	+	+	-
778	299	M	M 5	+	+	-
1 909	565	M	M 6	+	+	-
2 119	705	M	M 7	+	+	-
2 124	706	M	M 8	+	+	-
2 186	690	M	M 9	+	+	-
2 109	703	F	F 1	+	+	-
2 117	704	F	F 2	+	+	-
2 159	664	F	F 3	+	+	-
5 369	1 099	F	F 4	+	+	-
5 414	1 105	F	F 5	+	+	-
5 418	1 106	F	F 6	+	+	-
5 439	1 111	F	F 7	+	+	-
5 519	1 119	F	F 8	+	+	-
5 525	1 120	F	F 9	+	+	-

Table 3

SEROLOGICAL SURVEILLANCE OF CHICKEN FARMS
USING ENZYME-LINKED IMMUNOSORBENT ASSAY
(ELISA)

No	Place	Number of samples	Number of positive sera	Per cent of positive sera
1	Hevel Lahish	836	184	22
2	Tel-Aviv district	68	11	16
3	Jerusalem district	43	4	9
4	Netania	85	26	31
5	Rehovot	23	1	4
6	Gedera	15	0	0
7	Negev	105	39	37
8	Golan plateau	27	4	15
	Total	1202	269	22

SEROLOGICAL SURVEILLENCE OF CHICKEN FARMS
[HEMAGGLUTINATION INHIBITION (HI) TEST]

Place	Number of samples	Number of positive sera*	Per cent of positive sera
Hevel Lahish	651	86	13
Tel-Aviv district	108	9	8
Netania	75	1	1
Rehovot	52	1	2
Negev	31	3	10
Golan plateau	41	0	0
Total	958	100	10

* Positive HI titer > 1:20

Table 4 Hemagglutination-elution pattern of PMV-2 viruses

Viruses	Non eluters	Partial eluters	Moderate eluters	Fast eluters
Reference	Yucaipa PLOC Italy	Robin England Indonesia/3589 Indonesia/3650 Kenya		Haneda Bangor
Israeli	Ty/Kfar-Hess/7 Ty/Kfar-Hess/10B Ty/Burgata Ty/Sde-Yoav/15/T Ty/Nahla/687	Ty/Beit-Shearim/170 Ty/Sde-Yoav/15/C Ty/Kfar-Harutz Ty/Bear Tuvia/676 Ty/Ein Tsurim Ty/Tsora Ty/Talmi-Eliel Ty-Beit-Yitzhak Ty/Rosh-Tsurim Ty/Hatsav/6/T Ty/Hatsav/8/T	Mal/Ramon Ty/Sde Moshe Ty/Shalva Ch/Hatsav Coot/Beit Alfa Ty/Kfar-Hess/10A Coot/Shluhot Ch/Mishmeret/803 Ty/Bear-Tuvia/752 Ty/Neot Mordehai Ty/Hatsav/4/T Ty/Beit Shearim/595	Cegret/Ramat Gan Ty/Beit-Herut Ch/Mishmeret/589 Ty/Nahla/812 Ch/Petahia

Table 5 Cross HI test with reference PMV 2 viruses

Antisera	Viruses											
	Yucaipa	Robin	PLOC	Haneda	Bangor	England	Italy	Indonesia 3589	Indonesia 3650	Kenya	Ty/ Kfar Hess 10B	Ch/ Hatsav
Yucaipa	0	0.13 ± 0.24 $n = 4$ $P = 0.35$	1.37 ± 0.17 $n = 6$ $P > 0.99$	1.02 ± 0.21 $n = 9$ $P > 0.99$	1.59 ± 0.51 $n = 4$ $P = 0.95$	0.96 ± 0.74 $n = 4$ $P = 0.72$	0.94 ± 0.35 $n = 4$ $P = 0.93$	0.34 ± 0.17 $n = 3$ $P = 0.82$	0.39 ± 0.39 $n = 3$ $P = 0.58$	1.19 ± 0.21 $n = 4$ $P = 0.99$	0.89 ± 0.24 $n = 11$ $P = 0.99$	0.46 ± 0.23 $n = 6$ $P = 0.90$
Robin	3.38 ± 0.46 $n = 4$ $P > 0.99$	0	2.01 ± 0.01 $n = 2$ $P > 0.99$	1.89 ± 0.45 $n = 3$ $P = 0.95$	2.22 ± 0.35 $n = 3$ $P > 0.96$	2.57 ± 0.30 $n = 3$ $P > 0.96$	1.63 ± 0.39 $n = 3$ $P = 0.95$	2.06 ± 0.12 $n = 3$ $P > 0.96$	1.35 ± 0.32 $n = 3$ $P = 0.95$	2.23 ± 0.45 $n = 3$ $P > 0.99$	1.73 ± 0.71 $n = 8$ $P = 0.95$	2.03 ± 0.57 $n = 4$ $P = 0.96$
PLOC	1.88 ± 0.30 $n = 6$ $P > 0.99$	0.95 ± 0.76 $n = 3$ $P = 0.65$	0	0.32 ± 0.27 $n = 7$ $P = 0.72$	0.66 ± 0.17 $n = 4$ $P = 0.97$	0.62 ± 0.33 $n = 3$ $P = 0.82$	0.54 ± 0.52 $n = 4$ $P = 0.61$	0.08 ± 0.23 $n = 4$ $P = 0.22$	−0.26 ± 0.91 $n = 3$ $P = 0.21$	1.31 ± 0.63 $n = 3$ $P = 0.83$	0.48 ± 0.50 $n = 10$ $P = 0.66$	0.19 ± 0.50 $n = 6$ $P = 0.29$
Haneda	2.54 ± 0.29 $n = 9$ $P > 0.99$	1.68 ± 0.29 $n = 3$ $P = 0.97$	2.38 ± 0.20 $n = 7$ $P > 0.99$	0	2.40 ± 0.56 $n = 4$ $P = 0.98$	1.44 ± 0.22 $n = 3$ $P = 0.98$	1.42 ± 0.20 $n = 4$ $P > 0.99$	1.01 ± 0.17 $n = 3$ $P = 0.99$	0.79 ± 0.83 $n = 4$ $P = 0.54$	2.50 ± 0.21 $n = 4$ $P > 0.99$	2.02 ± 0.18 $n = 12$ $P > 0.99$	1.50 ± 0.24 $n = 8$ $P > 0.99$
Ty/Kfar Hess 10B	2.06 ± 0.51 $n = 9$ $P > 0.99$	1.28 ± 0.47 $n = 5$ $P = 0.95$	1.23 ± 0.68 $n = 8$ $P = 0.88$	1.01 ± 0.59 $n = 9$ $P = 0.87$	1.68 ± 0.61 $n = 5$ $P = 0.95$	3.22 ± 1.05 $n = 5$ $P = 0.96$	0.19 ± 0.46 $n = 3$ $P = 0.27$	0.59 ± 1.14 $n = 3$ $P = 0.33$	−0.66 ± 0.50 $n = 3$ $P = 0.68$	−0.14 ± 0.56 $n = 3$ $P = 0.14$	0	0.75 ± 0.68 $n = 9$ $P = 0.70$
Ch/Hatsav	2.26 ± 0.57 $n = 11$ $P > 0.99$	1.97 ± 0.79 $n = 6$ $P = 0.95$	0.98 ± 0.43 $n = 10$ $P = 0.95$	1.47 ± 0.61 $n = 11$ $P = 0.97$	0.67 ± 0.28 $n = 7$ $P = 0.95$	1.50 ± 0.41 $n = 8$ $P = 0.99$	−0.18 ± 0.30 $n = 6$ $P = 0.42$	0.10 ± 0.72 $n = 8$ $P = 0.08$	1.40 ± 0.52 $n = 8$ $P = 0.97$	1.40 ± 0.52 $n = 4$ $P = 0.77$	1.23 ± 0.80 $n = 7$ $P = 0.15$	0.17 ± 0.87 0

Upper figure d in each cell is the mean value (with standard error) of $\log_2$ of the ratio between homologous and heterologous HI titers when a given antiserum was the same for the two viruses compared n is the number of experiments for each pair of compared viruses P is Student's coefficient of probability for the difference d found for a given number of experiments n it is expressed as a percentage of probability of the zero hypothesis i e the probability that the difference d itself differs significantly from zero The reliability of the d values (their difference from zero) was accepted as significant if it was in agreement with Fischer's 2nd criterion of probability namely 95% probability ($P = 0.95$)

The values of d which differ significantly from zero ($P \geq 0.95$) are bold faced

Table 6 Cross HI test with Israeli strains isolated in 1979

Antisera	Viruses									
	Mal/ Ramon	Ty/ Kfar Hess/7	Ty/ Kfar Hess/10A	Ty/ Kfar Hess/10B	Ty/ Burgata	Ty/ Beit Shearim 170	Ty/ Sde Yoav 15/T	Ty/ Sde Yoav 15/C	Cegret/ Ramat Gan	M ± SD
Yucaipa	1.34 ± 0.35 $n = 4$ $P = 0.97$	1.42 ± 0.24 $n = 3$ $P = 0.97$	−0.14 ± 0.41 $n = 3$ $P = 0.21$	1.12 ± 0.32 $n = 5$ $P = 0.98$	0.86 ± 0.25 $n = 4$ $P = 0.96$	0.28 ± 0.19 $n = 3$ $P = 0.73$	0.19 ± 0.30 $n = 8$ $P = 0.43$	0.04 ± 1.10 $n = 4$ $P \leqslant 0.06$	−0.28 ± 0.27 $n = 3$ $P = 0.58$	0.54 ± 0.22
Robin	2.60 ± 0.64 $n = 4$ $P = 0.97$	2.13 ± 0.40 $n = 4$ $P = 0.95$	1.01 ± 0.53 $n = 3$ $P = 0.80$	2.04 ± 0.53 $n = 4$ $P = 0.97$	2.40 ± 0.52 $n = 4$ $P = 0.98$	2.00 ± 0.85 $n = 4$ $P = 0.99$	1.51 ± 0.75 $n = 8$ $P = 0.99$	1.90 ± 0.57 $n = 4$ $P = 0.86$	ND	1.95 ± 0.18
PLOC	1.08 ± 0.63 $n = 3$ $P = 0.77$	1.06 ± 0.16 $n = 3$ $P = 0.99$	−0.71 ± 0.70 $n = 4$ $P = 0.61$	1.04 ± 0.58 $n = 3$ $P = 0.79$	1.22 ± 0.67 $n = 4$ $P = 0.84$	ND	−0.20 ± 1.30 $n = 6$ $P = 0.08$	0.40 ± 1.28 $n = 4$ $P = 0.22$	0.60 ± 0.33 $n = 3$ $P = 0.79$	0.56 ± 0.25
Haneda	1.61 ± 0.77 $n = 4$ $P > 0.87$	3.02 ± 0.02 $n = 2$ $P > 0.96$	0.83 ± 0.34 $n = 4$ $P = 0.90$	2.15 ± 0.19 $n = 6$ $P > 0.99$	1.92 ± 0.60 $n = 4$ $P = 0.95$	1.69 ± 0.46 $n = 4$ $P = 0.96$	0.99 ± 0.60 $n = 8$ $P = 0.85$	1.46 ± 0.26 $n = 4$ $P = 0.99$	0.08 ± 0.08 $n = 3$ $P = 0.50$	1.53 ± 0.28
Ty/Kfar Hess 10B	−0.13 ± 0.45 $n = 3$ $P = 0.21$	0.46 ± 0.37 $n = 3$ $P = 0.65$	0.18 ± 0.40 $n = 3$ $P = 0.33$	0	−0.72 ± 0.71 $n = 4$ $P = 0.61$	0.26 ± 0.13 $n = 3$ $P = 0.82$	−0.23 ± 0.50 $n = 10$ $P = 0.37$	0.29 ± 1.12 $n = 8$ $P = 0.23$	ND	0.02 ± 0.15
4Ch/Hatsav	0.77 ± 0.66 $n = 10$ $P = 0.74$	1.40 ± 0.98 $n = 3$ $P = 0.70$	−1.01 ± 0.34 $n = 5$ $P = 0.96$	0.17 ± 0.87 $n = 7$ $P = 0.15$	0.27 ± 0.73 $n = 7$ $P = 0.30$	ND	−0.47 ± 0.64 $n = 8$ $P = 0.49$	−0.44 ± 1.52 $n = 8$ $P = 0.23$	0.27 ± 0.57 $n = 3$ $P = 0.33$	0.12 ± 0.27

The designations are as for Table 2
M ± SD—average d with standard deviation

Table 7 Cross HI test with Israeli strains isolated in 1980

Antisera	Viruses														
	Ty/ Beit Herut	Ch/ Mishmeret 589	Ty/ Neot Mordahai	Ty/ Beit Shearim 595	Ty/ Rosh Tsurim	Ty/ Bear Tuvia 676	Ty/ Kfar Harutz	Ty/ Nahla 687	Ch/ Hatsav	Ty/ Bear Tuvia 752	Ch/ Mishmeret 803	Ty/ Shalva	Ty/ Nahla 812	Ty/ Sde Moshe	Coot Shluhot	M ± SD
Yucaipa	−0.52 ± 0.66 n = 3 P = 0.49	0.57 ± 0.35 n = 4 P = 0.79	0.60 ± 0.50 n = 3 P = 0.65	0.17 ± 0.38 n = 3 P = 0.27	0.66 ± 0.93 n = 3 P = 0.44	−0.22 ± 0.32 n = 3 P = 0.44	0.63 ± 0.39 n = 6 P = 0.83	1.32 ± 0.42 n = 4 P > 0.98	0.46 ± 0.23 n = 6 P = 0.90	−0.06 ± 0.54 n = 4 P = 0.54	0.06 ± 0.39 n = 3 P = 0.21	−0.19 ± 0.42 n = 5 P = 0.30	−0.26 ± 0.24 n = 3 P = 0.61	0.10 ± 0.27 n = 3 P = 0.27	0.09 ± 0.36 n = 4 P = 0.22	0.23 ± 0.12
Robin	0.62 ± 0.73 n = 3 P = 0.8[illegible]	2.42 ± 0.78 n = 4 P = 0.95	1.22 ± 0.29 n = 3 P = 0.95	1.80 ± 0.35 n = 3 P = 0.96	1.55 ± 0.50 n = 4 P = 0.95	0.43 ± 0.27 n = 3 P = 0.75	1.92 ± 0.48 n = 4 P = 0.96	3.37 ± 1.08 n = 4 P = 0.95	2.03 ± 0.57 n = 3 P = 0.96	1.11 ± 0.26 n = 3 P = 0.95	0.56 ± 1.28 n = 3 P = 0.44	1.36 ± 1.08 n = 4 P = 0.70	0.65 ± 0.55 n = 3 P = 0.65	0.29 ± 0.14 n = 3 P = 0.83	1.30 ± 0.31 n = 3 P = 0.95	1.38 ± 0.22
PLOC	−0.18 ± 0.55 n = 3 P = 0.21	1.13 ± 0.51 n = 4 P = 0.89	0.20 ± 0.29 n = 3 P = 0.44	1.33 ± 0.39 n = 4 P = 0.96	0.68 ± 0.84 n = 3 P = 0.49	−0.23 ± 0.17 n = 3 P = 0.70	0.52 ± 0.60 n = 4 P = 0.[illegible]4	1.18 ± 0.74 n = 3 P = 0.74	0.19 ± 0.50 n = 6 P = 0.29	0.98 ± 0.86 n = 3 P = 0.61	0.71 ± 0.78 n = 3 P = 0.54	−0.69 ± 0.26 n = 3 P = 0.89	−0.31 ± 0.22 n = 3 P = 0.70	−0.26 ± 0.15 n = 3 P = 0.77	0.18 ± 0.26 n = 3 P = 0.41	0.30 ± 0.16
Haneda	0.40 ± 0.70 n = 3 P = 0.39	1.80 ± 0.23 n = 7 P > 0.99	1.01 ± 0.23 n = 6 P = 0.99	1.77 ± 0.29 n = 3 P = 0.99	1.84 ± 0.34 n = 4 P = 0.99	0.08 ± 0.48 n = 3 P = 0.14	1.80 ± 0.26 n = 6 P > 0.99	3.01 ± 0.82 n = 5 P = 0.98	1.50 ± 0.24 n = 8 P ≥ 0.99	0.94 ± 0.31 n = 6 P = 0.97	1.52 ± 0.36 n = 4 P = 0.97	1.22 ± 0.41 n = 6 P = 0.97	−0.56 ± 0.75 n = 3 P = 0.44	0.08 ± 0.58 n = 3 P = 0.07	1.15 ± 0.34 n = 4 P = 0.96	1.17 ± 0.23
Ty/Kfar Hess 10B	−1.47 ± 1.00 n = 3 P = 0.73	0.97 ± 1.08 n = 7 P = 0.61	0.14 ± 0.71 n = 4 P = 0.15	0.65 ± 1.05 n = 6 P = 0.42	0.00 ± 1.00 n = 3	−1.90 ± 0.78 n = 3 P = 0.87	0.07 ± 0.92 n = 8 P = 0.08	−0.60 ± 0.66 n = 5 P = 0.58	0.75 ± 0.68 n = 9 P = 0.70	−0.96 ± 0.58 n = 5 P = 0.84	−0.60 ± 0.72 n = 4 P = 0.52	−1.55 ± 0.66 n = 7 P = 0.94	−0.30 ± 0.69 n = 5 P = 0.29	−0.96 ± 1.00 n = 3 P = 0.58	0.06 ± 0.55 n = 5 P = 0.07	−0.38 ± 0.22
Ch/Hatsav	−0.40 ± 0.68 n = 3 P = 0.39	0.21 ± 0.80 n = 8 P = 0.23	0.21 ± 1.18 n = 7 P = 0.15	−0.59 ± 0.38 n = 8 P = 0.85	−0.65 ± 1.12 n = 5 P = 0.42	−1.59 ± 0.95 n = 5 P = 0.84	0.33 ± 1.07 n = 6 P = 0.22	1.04 ± 1.26 n = 7 P = 0.55	0	−0.08 ± 0.51 n = 9 P = 0.15	−0.13 ± 0.62 n = 8 P = 0.15	−1.06 ± 1.33 n = 6 P = 0.54	−2.32 ± 0.18 n = 4 P > 0.99	−1.67 ± 0.32 n = 5 P > 0.99	−0.88 ± 0.46 n = 7 P = 0.89	−0.54 ± 0.24

Table 8 Cross HI test with Israeli strains isolated in 1981

Antisera	Viruses									M ± SD
	Ch/ Petahia	Ty/ Ein Tsurim	Ty/ Tsora	Ty/ Talmi Eliel	Ty/ Beit Yitzhak	Ty/ Hatsav T4	Coot/ Beit Alfa	Ty/ Hatsav T6	Ty/ Hatsav T8	
Yucaipa	1 17 ± 0 38 $n = 4$ $P = 0\ 95$	ND	−0 16 ± 0 50 $n = 4$ $P = 0\ 22$	−0 40 ± 0 32 $n = 3$ $P = 0\ 65$	−0 77 ± 0 71 $n = 3$ $P = 0\ 61$	−0 23 ± 0 20 $n = 3$ $P = 0\ 77$	0 43 ± 0 41 $n = 6$ $P = 0\ 64$	0 10 ± 0 32 $n = 6$ $P = 0\ 22$	0 27 ± 0 12 $n = 5$ $P = 0\ 91$	0 05 ± 0 21
Robin	2 63 ± 0 54 $n = 3$ $P = 0\ 96$	2 96 ± 0 70 $n = 3$ $P = 0\ 95$	2 19 ± 0 71 $n = 4$ $P = 0\ 95$	0 18 ± 0 11 $n = 3$ $P = 0\ 75$	−0 18 ± 0 20 $n = 3$ $P = 0\ 54$	1 19 ± 0 27 $n = 3$ $P = 0\ 95$	2 00 ± 0 60 $n = 4$ $P = 0\ 95$	1 33 ± 0 26 $n = 6$ $P > 0\ 99$	2 55 ± 0 57 $n = 3$ $P = 0\ 95$	1 65 ± 0 55
PLOC	1 89 ± 0 67 $n = 3$ $P = 0\ 89$	3 21 ± 0 54 $n = 3$ $P > 0\ 96$	1 34 ± 0 67 $n = 4$ $P = 0\ 88$	−0 06 ± 0 23 $n = 3$ $P = 0\ 21$	0 29 ± 0 60 $n = 3$ $P = 0\ 48$	−0 36 ± 0 52 $n = 3$ $P = 0\ 44$	0 31 ± 0 29 $n = 3$ $P = 0\ 61$	−0 03 ± 0 42 $n = 6$ $P = 0\ 08$	0 00 ± 0 30 $n = 4$	0 73 ± 0 42
Haneda	3 01 ± 0 19 $n = 3$ $P > 0\ 96$	2 78 ± 0 66 $n = 3$ $P = 0\ 95$	1 88 ± 0 59 $n = 4$ $P = 0\ 95$	0 93 ± 0 13 $n = 3$ $P > 0\ 96$	0 32 ± 0 66 $n = 3$ $P = 0\ 33$	1 57 ± 0 59 $n = 5$ $P = 0\ 95$	1 81 ± 0 20 $n = 6$ $P > 0\ 99$	1 41 ± 0 44 $n = 6$ $P = 0\ 98$	0 88 ± 0 23 $n = 7$ $P = 0\ 99$	1 62 ± 0 29
Ty/Kfar Hess 10B	1 65 ± 0 65 $n = 6$ $P = 0\ 95$	3 40 ± 0 61 $n = 3$ $P = 0\ 96$	0 21 ± 1 47 $n = 5$ $P = 0\ 07$	−0 21 ± 0 97 $n = 5$ $P = 0\ 15$	−2 31 ± 1 61 $n = 3$ $P = 0\ 70$	1 18 ± 0 84 $n = 3$ $P = 0\ 70$	1 43 ± 0 62 $n = 9$ $P = 0\ 95$	1 61 ± 1 50 $n = 6$ $P = 0\ 68$	−0 27 ± 0 78 $n = 3$ $P = 0\ 21$	0 74 ± 0 54
Ch/Hatsav	1 45 ± 0 54 $n = 5$ $P = 0\ 95$	1 02 ± 0 30 $n = 7$ $P = 0\ 99$	0 14 ± 0 50 $n = 7$ $P = 0\ 23$	−0 94 ± 1 06 $n = 5$ $P = 0\ 58$	0 42 ± 2 20 $n = 5$ $P = 0\ 15$	0 17 ± 0 52 $n = 4$ $P = 0\ 22$	0 71 ± 0 73 $n = 8$ $P = 0\ 65$	0 06 ± 0 34 $n = 12$ $P = 0\ 15$	0 17 ± 0 14 $n = 10$ $P = 0\ 74$	0 36 ± 0 23

The designations are as for Table 3

Table 9 List of the APMV-2 strains isolated in 1994

	VIRUSES	ABBREVIATIONS
1	APMV-2/Chicken/Israel, Hadera/1545/94 3	Ch/Hadera/1545
2	APMV-2/Chicken/Israel, Hadera/2058/94 3	Ch/Hadera/2058
3	APMV-2/Turkey/Israel, Hatsav/2470/94 4	Ty/Hatsav/2470
4	APMV-2/Turkey/Israel, Hatsav/1441/94 5	Ty/Hatsav/1441
5	APMV-2/Turkey/Israel, Hatsav/1442/94 5	Ty/Hatsav/1442
6	APMV-2/Turkey/Israel, Hatsav/822/94 5	Ty/Hatsav/822
7	APMV-2/Turkey/Israel, Hatsav/2986/94 5	Ty/Hatsav/2986
8	APMV-2/Turkey/Israel, Hatsav/2264/94 5	Ty/Hatsav/2264
9	APMV-2/Turkey/Israel, Ramot/1852/94 5	Ty/Ramot/1852
10	APMV-2/Turkey/Israel, Ramot/513/94 5	Ty/Ramot/513
11	APMV-2/Turkey/Israel, Ramot/1750/94 5	Ty/Ramot/1750
12	APMV-2/Turkey/Israel, Ramot/1956/94 5	Ty/Ramot/1956
13	APMV-2/Turkey/Israel, Ramot/37/94 5	Ty/Ramot/37
14	APMV-2/Turkey/Israel, Ramot/2369/94 5	Ty/Ramot/2369
15	APMV-2/Turkey/Israel, Ramot/40118/94 5	Ty/Ramot/40118
16	APMV-2/Turkey/Israel, Herut/2161/94 7	Ty/Herut/2161
17	APMV-2/Duck/Israel, Herut/1344/94 7	Dk/Herut/1344
18	APMV-2/Duck/Israel, Herut/1750/94 7	Dk/Herut/1750
19	APMV-2/Imperial sandgrouse, T-A Zoo/3191/94 5	Imp sgr/Zoo

Table 10 Thermostability of the APMV-2 strains isolated in 1979-81

	VIRUSES	HA activity	
		Native	Heated
1	APMV-2/Mallard/Israel, Ramon/4/T/79 1	64	48
2	APMV-2/Turkey/Israel, Kfar-Hess/7/T/79 3	64	<2
3	APMV-2/Turkey/Israel, Kfar-Hess/10A/B/79 3	64	2
4	APMV-2/Turkey/Israel, Kfar-Hess/10B/B/79 3	32	2
5	APMV-2/Turkey/Israel, Beit-Shearim/170/L/79 4	128	<2
6	APMV-2/Turkey/Israel, Sde-Yoav/15/C/79 9	16	4
7	APMV-2/Chicken/Israel, Mishmeret/589/L/80 10	64	<2
8	APMV-2/Turkey/Israel, Rosh-Tsurim/596/80 10	16	<2
9	APMV-2/Turkey/Israel, Bear-Tuvia/676/B/80 10	32	<2
10	APMV-2/Turkey/Israel, Kfar-Harutz/685/B/80 10	16	2
11	APMV-2/Turkey/Israel, Nahla/687/B/80 10	256	<2
12	APMV-2/Turkey/Israel, Bear-Tuvia/752/B/80 11	16	16
13	APMV-2/Chicken/Israel, Mishmeret/803/K/80 11	16	<2
14	APMV-2/Turkey/Israel, Nahla/812/80	16	<2
15	APMV-2/Turkey/Israel, Tsora/830/B/81 1	32	<2
16	APMV-2/Turkey/Israel, Talmi-Ehiel/837/B/81 1	64	<2
17	APMV-2/Turkey/Israel, Hatsav/4T/81 1	64	4
18	APMV-2/Turkey/Israel, Hatsav/6T/81 3	128	<2

Table 11 Thermostability of the APMV-2 strains isolated in 1994

	VIRUSES	HA activity	
		Native	Heated
1	APMV-2/Chicken/Israel, Hadera/1545/94 3	32	<2
2	APMV-2/Chicken/Israel, Hadera/2058/94 3	256	256
3	APMV-2/Turkey/Israel, Hatsav/2470/94 4	64	64
4	APMV-2/Turkey/Israel, Hatsav/1441/94 5	64	<2
5	APMV-2/Turkey/Israel, Hatsav/1442/94 5	64	<2
6	APMV-2/Turkey/Israel, Hatsav/822/94 5	16	<2
7	APMV-2/Turkey/Israel, Ramot/1852/94 5	64	<2
8	APMV-2/Turkey/Israel, Ramot/513/94 5	32	32
9	APMV-2/Turkey/Israel, Ramot/1750/94 5	256	256
10	APMV-2/Turkey/Israel, Ramot/1956/94 5	64	<2
11	APMV-2/Turkey/Israel, Ramot/2369/94 5	128	<2
12	APMV-2/Turkey/Israel, Ramot/40118/94 5	128	128
13	APMV-2/Duck/Israel Herut/1344/94 7	32	<2
14	APMV-2/Duck/Israel, Herut/1750/94 7	256	256
15	APMV-2/Imperial sandgrouse, T-A Zoo/3191/94 5	32	<2

Table 12 Hemagglutination-elution pattern of the APMV-2 strains isolated in 1994

	VIRUSES	RATE OF ELUTION
1	APMV-2/Chicken/Israel, Hadera/1545/94 3	Non-eluter
2	APMV-2/Chicken/Israel, Hadera/2058/94 3	Non-eluter
3	APMV-2/Turkey/Israel, Hatsav/1441/94 5	Non-eluter
4	APMV-2/Turkey/Israel, Hatsav/1442/94 5	Fast eluter
5	APMV-2/Turkey/Israel, Hatsav/822/94 5	Non-eluter
6	APMV-2/Turkey/Israel, Ramot/1852/94 5	Fast eluter
7	APMV-2/Turkey/Israel, Ramot/513/94 5	Non-eluter
8	APMV-2/Turkey/Israel, Ramot/1750/94 5	Non-eluter
9	APMV-2/Turkey/Israel, Ramot/1956/94 5	Fast eluter
10	APMV-2/Turkey/Israel, Ramot/37/94 5	Moderate eluter
11	APMV-2/Turkey/Israel, Ramot/2369/94 5	Fast eluter
12	APMV-2/Turkey/Israel, Ramot/40118/94 5	Non-eluter
13	APMV-2/Duck/Israel, Herut/1344/94 7	Fast eluter
14	APMV-2/Duck/Israel, Herut/1750/94 7	Non-eluter
15	APMV-2/Imperial sandgrouse, T-A Zoo/3191/94 5	Non-eluter

Table 13

Identification of the isolates as APMV-4

SEROTYPE*	REFERENCE VIRUSES	HI TEST with ISOLATES		
		C-9	C-25	T-27
APMV-1	APMV-1/NDV/LaSota	20	10	40
APMV-2	APMV-2/chicken/California/Yucaipa/56	<10	<10	<10
APMV-3a	APMV-3/turkey/Wisconsin/67	<10	<10	<10
APMV-3b	APMV-3/parakeet/Netherlands/449/75	10	10	10
APMV-4	APMV-4/duck/Hong Kong/D3/75	320	320	160
APMV-6	APMV-6/duck/Hong Kong/D199/77	<10	<10	<10
APMV-7	APMV-7/dove/Tennessee/4/75	<10	<10	<10
APMV-8	APMV-8/goose/Delaware/1053/76	<10	<10	<10
APMV-9	APMV-9/duck/New York/22/80	<10	<10	<10
APMV-?	APMV-?/pigeon/Otaru/76	<10	<10	<10

* Serotype APMV-5 (APMV-5/budgerigar/Kunitachi) was not used in the studies since it was not available

Table 14 HEMAGGLUTINATION INHIBITION CROSS REACTION

ANTISERA	VIRUSES				
	NDV	APMV-4	C-9	C-25	T-27
NDV	0	3 23 ± 0 98 n = 5 P = 0 97	3 53 ± 0 91 n = 5 P = 0 97	3 35 ± 0 78 n = 5 P = 0 99	1 18 ± 0 54 n = 5 P = 0 91
APMV-4	4 58 ± 0 98 n = 3 P > 0 99	0	-2 38 ± 1 12 n = 5 P = 0 91	-0 33 ± 1 26 n = 6	-1 25 ± 1 53 n = 6
C-9	0 46 ± 1 16 n = 5	0 45 ± 1 46 n = 6	0	0 91 ± 0 40 n = 6	0 04 ± 0 41 n = 5
C-25	0 01 ± 0 55 n = 5	0 03 ± 0 83 n = 5	-0 06 ± 0 69 n = 6	0	-0 63 ± 0 72 n = 6
T-27	1 10 ± 0 73 n = 5 P = 0 79	1 05 ± 1 37 n = 6	-0 18 ± 0 21 n = 6	0 80 ± 0 58 n = 6	0

* Upper figure *d* is a mean value (with standard error) of log of the ratio between homologous and heterologous HI titres when a given antiserum was the same for the two compared viruses *n* is a number of experiments for each pair of compared viruses *P* is Student s coefficient of probability for the found difference *d* for a given number of experiments *n* it is expressed as a percentage of probability of the zero hypothesis i e the probability that the difference *d* itself differs significantly from zero The reliability of the *d* values (their difference from zero) was accepted as significant if it was in agreement with Fisher s second criterion of probability namely 95% probability ($P = 0\ 95$) When there was no cross reactivity between viruses the *d* value is expressed as infinity (∞)

Table 15 Recovery of separate viruses from mixed isolates by their treatment with APMV reference antisera.

ISOLATE	Antiserum used for the treatment of the isolate	HI with APMV antisera*		
		anti-NDV	anti-APMV-4	anti-APMV-2
51-152	anti-NDV	<1 20	1 320	-
	anti-APMV-4	1 640	<1 20	-
28-83	anti-NDV	<1 20	-	1 320
	anti-APMV-2	1 160	-	<1 20
29-86	anti-NDV	<1 20	-	1 320
	anti-APMV-2	1 480	-	<1 20

* after passaging of the antiserum-treated isolate

Table 16

CLASSIFICATION OF ANTI-NDV MONOCLONAL ANTIBODIES BY THEIR REACTIVITY WITH PROTEINS OF STRAIN AUSTRALIA-VICTORIA IN ELISA AND ANTIGEN-CAPTURE ELISA TESTS

No	MONOCLONAL ANTIBODY CLONE	ELISA TITER	MABs REACTIVITY IN ANTIGEN-CAPTURE ELISA (per cent) HN (30C2)*	F (1F2)**	M (15C9)***
1	34C10	1.10000	100	16	0
2	42B3	1:5000	100	0	0
3	28E7	1 5000	100	0	0
4	24C8	1 1000	100	0	0
5	3E2	1 5000	100	0	0
6	14F11	1 5000	100	0	2
7	28C10	1 10000	100	4	0
8	30C2	1 5000	100	6	0
9	3E3	1 5000	100	4	0
10	2G7	1 1000	100	0	0
11	3B5	1 1000	4	100	1
12	6E4	1 2000	6	100	2
13	6E6	1 2000	3	100	3
14	1F3	1 10000	10	100	0
15	1D2	1 1000	3	100	5
16	1F10	1 5000	0	100	0
17	4F11	1 5000	7	100	0
18	15C9	1 10000	0	0	100
19	38E10	1·10000	3	2	100
20	15F2	1 1000	0	0	100
21	25C9	1·10000	3	4	100
22	43C3	1 5000	0	0	100
23	13D7	1 1000	0	0	100

* 30C2 is a representative MAB from a panel qulified as anti-HN by RIP test

** 1F2 is a representative MAB from a panel qulified as anti-F by RIP test

*** 15C9 is a representative MAB from a panel qulified as anti-M by RIP test

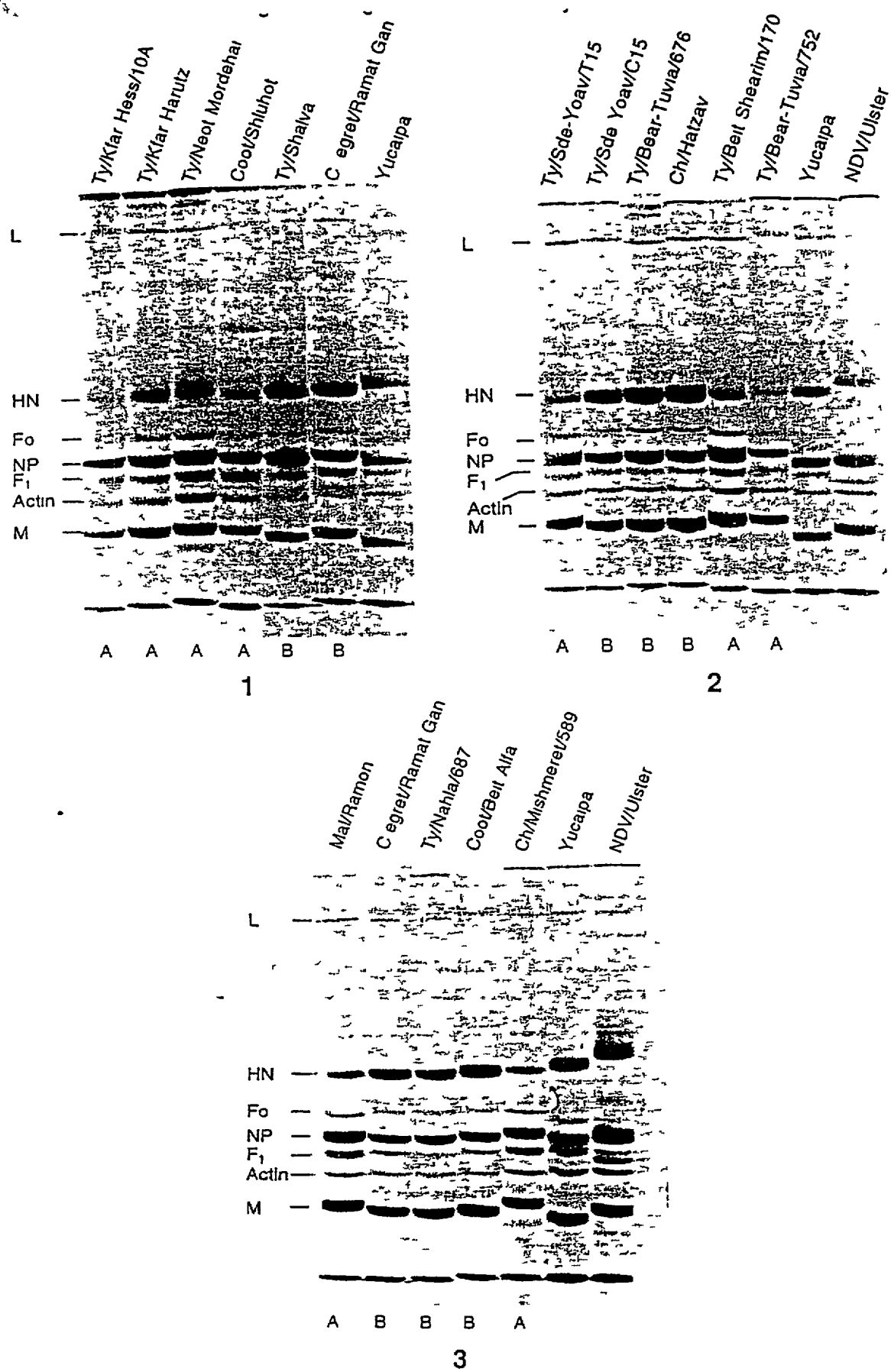

Fig 1 Structural polypeptides of the PMV-2 virus isolates 1 2, 3—parts of the figure representing different experiments, L, HN, NP, F_1 and M—structural viral polypeptides, Act—actin A and B are designations of the two groups into which the Israeli isolates could be distributed

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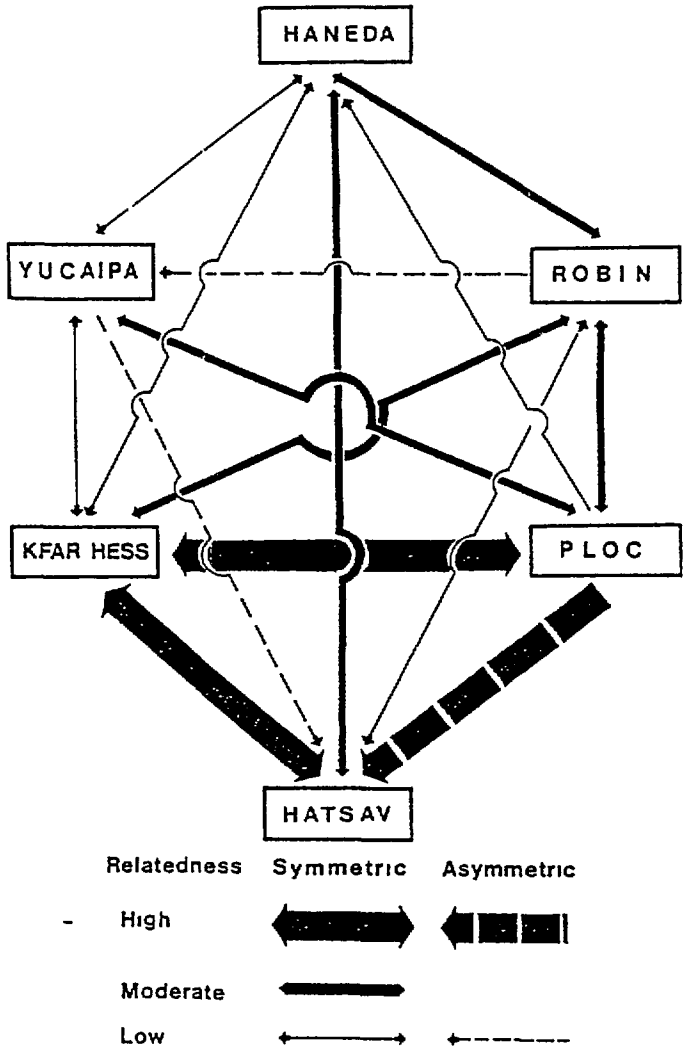

Fig 2 Graphic display of antigenic relationships between prototype and Israeli reference PMV-2 viruses

Fig 3

Isolation of HA agents from domestic and feral birds in Kenya

No	ISOLATE No	AVIAN SPECIES	ORGAN/ SWAB	PLACE of ISOLATION	DATE of ISOLATION	PASSAGE No
1	KRC62	Chicken	Cloaca	KARIOKOR	24 06 94	2
2	KRC63				24 06 94	2
3	KRC64				24 06 94	3
4	KRC65				24 06 94	2
5	KRC74b				24 06 94	2
6	KRC75				24 06 94	2
7	KRC81b				24 06 94	2
8	KRC83				24 06 94	2
9	KRC92				24 06 94	2
10	KRC93				24 06 94	3
11	KRC97				24 06 94	2
12	KRC98c				24 06 94	3
13	KRC101				07 07 94	2
14	KRC102				07.07 94	2
15	KRC103				07 07 94	2
16	KRC106i				07 07 94	2
17	KRC108				07 07 94	2
18	KRC109				07 07 94	2
19	KRC114				07 07 94	2
20	KPC12				07 07 94	2
21	KRC123				07 07 94	2
22	KRC125				07 07 94	2
23	KRC126c				07 07 94	2
24	KRC127b				07 07 94	3
25	KRC128				07 07 94	2
26	KPC131				07 07 94	2
27	KRC132b				07 07 94	3
28	KRC134				07 07 94	2
29	KRC136				07 07 94	2
30	KRC137				07 07 94	3
31	KRC139				07 07 94	2
32	KRC141				07 07 94	2
33	KRC143				07 07 94	2
34	KRC144				07 07 94	2
35	KRC149				07 07 94	2
36	KRC150				07 07 94	2
37	KRC151				08 07 94	1
38	KRC152a				08 07 94	3
39	KRC181				08 07 94	2
40	KRC184				08 07 94	2
41	KRC213				14 07 94	2
42	KRC221				14 07 94	2
43	KRC223				14 07 94	2
44	KRC236				14 07 94	1
45	KRC237				14 07 94	2

Fig 3 (Continuation)

Isolation of HA agents from domestic and feral birds in Kenya

46	KRC238	Chicken	Cloaca	KARIOKOR	14	07	94	2
47	KRC239				14	07	94	1
48	KRC242				14	07	94	2
49	KRC244				14	07	94	2
50	KRC250				14	07	94	2
51	KRC251				19	07	94	3
52	KRC252a				19	07	94	4
53	KRC257				19	07	94	2
54	KRC263				19	07	94	1
55	KRC267b				19	07	94	4
56	KRC270				19	07	94	2
57	KRC279				19	07	94	2
58	KRC290				19	07	94	2
59	KRC322				21	07	94	2
60	KRC362				21	07	94	2
61	KRC457				09	08	94	3
62	KBC1			KABETE	18	09	94	2
63	A394a		Lung/Tra		15	06	94	2
64	A457		Marrow	KERICHO	17	07	94	3
65	A458a				17	07	94	3
66	A460				17	07	94	2
67	A462				17	07	94	4
68	A466		Lung/Tra	NAIROBI	18	07	94	2
69	A554		Marrow	NAKURU	12	08	94	2
70	47C		Cloaca	KERUGOYA	29	09	94	2
71	55C			NAKURU	29	09	94	2
72	62C			KERUGOYA	29	09	94	2
73	63C				29	09	94	2
74	66C				29	09	94	2
75	76D	Duck			29	09	94	2
76	DV141/92		Lung/Tra	KIAMBU	07	04	92	2
77	DV93/93		Lung/Tra		04	06	93	1
78	DV103/94		Lung/Tra		01	09	94	2
79	DV107/94		Lung/Tra		14	09	94	1
80	DV21/95		Cloaca		27	01	95	1
81	TD1	Duck		THIKA	29	09	94	2
82	TD2				29	09	94	1
83	TT1	Turkey			29	09	94	1
84	TT2				29	09	94	1
85	TT3				29	09	94	1
86	TT4				29	09	94	1
87	TC1	Chicken			29	09	94	1
88	TC2				29	09	94	1
89	TC3				29	09	94	1

Fig 3 (Continuation)

Isolation of HA agents from domestic and feral birds in Kenya

90	KWC1		KAWANGWARE	02	06	94	1
91	KWC3			02	06	94	1
92	KWC10			02	06	94	2
93	KWC11			02	06	94	2
94	KWC13			02	06	94	1
95	KWC19			02	06	94	1
96	KWC21			02	06	94	2
97	KWC22			02	06	94	1
98	KWC23			02	06	94	2
99	KWC30			02	06	94	2
100	KWC43			02	06	94	1
101	KLC2		KALOLENI	14	06	94	2
102	KLC3			14	06	94	2
103	KLC5			14	06	94	2
104	KLC15			14	06	94	1
105	KLC16			14	06	94	1
106	KLC17			14	06	94	2
107	KLC18			14	06	94	2
108	KLL19			14	06	94	2
109	KLC20			14	06	94	2
110	KLC21			14	06	94	2
111	KLC32			14	06	94	1
112	KLC33			14	06	94	2
113	WC1		WESTLANDS	18	06	94	2
114	WC2			18	06	94	3
115	WC3			18	06	94	2
116	WC5			18	06	94	1
117	WC8			18	06	94	2
118	WC10			18	06	94	1
119	WC15			18	06	94	2
120	WC16			18	06	94	2
121	WC20			18	06	94	2
122	WC21			18	06	94	2
123	WC24			18	06	94	2
124	WC26			18	06	94	2
125	WC28			18	06	94	2
126	KTC1	Turkey	NAIROBI	15	12	94	1
127	Cloacal	Duck	RUARAKA	20	11	94	2
128	LST	Chicken Lung/Tra/Lien	CHUKA	21	11	94	2
129	Trachea	Trachea	EMBU	20	11	94	2

Fig 3 (Continuation)

Isolation of HA agents from domestic and feral birds in Kenya

130	K1	Wild bird	Cloaca	15 02 95	2
131	K2			15 02 95	2
132	K3b			15 02 95	2
133	K6		'	15 02 95	2
134	K8	'		15 02 95	1
135	K9	'		15 02 95	2
136	K11	"		15 02 95	2
137	K12			15 02 95	1
138	K13	'		15 02 95	2
139	K14			15 02 95	2
140	K16			15 02 95	2

Fig 4 a

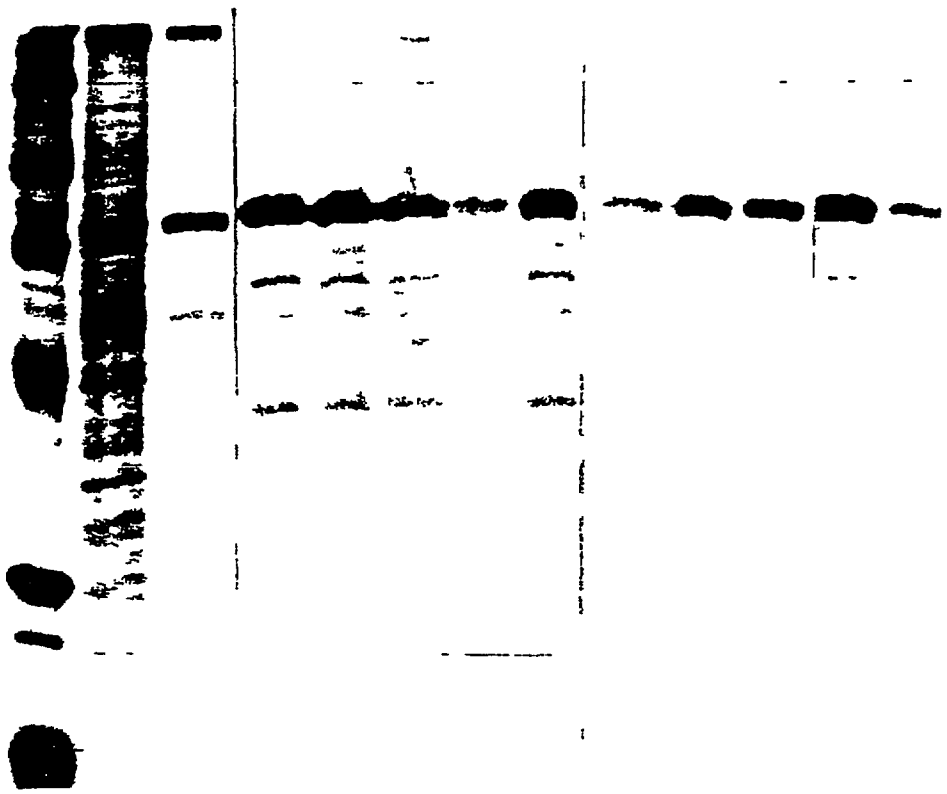

Fig 4a SDS-PAGE analysis of the NDV polypeptides (Australia-Victoria strain) immunoprecipitated by monoclonal atibodies (lane 1-10) from lysates of virus-infected BHK-21 cells labelled with [^{35}S]-methionine Lane (a) molecular weight markers; Lane (b) [^{35}S]-methionine-labelled virus-infected cell lysate, Lane (c) [^{3}H]-glucosamine-labelled virus-infected cell lysate. MAB clones: 1 - 34C10, 2 - 42B3, 3 - 28E7, 4 - 24C8, 5 - 3E2, 6 - 14F11, 7 - 28C10, 8 - 30C2, 9 - 3E3, 10 - 2G7.

Fig 4 b

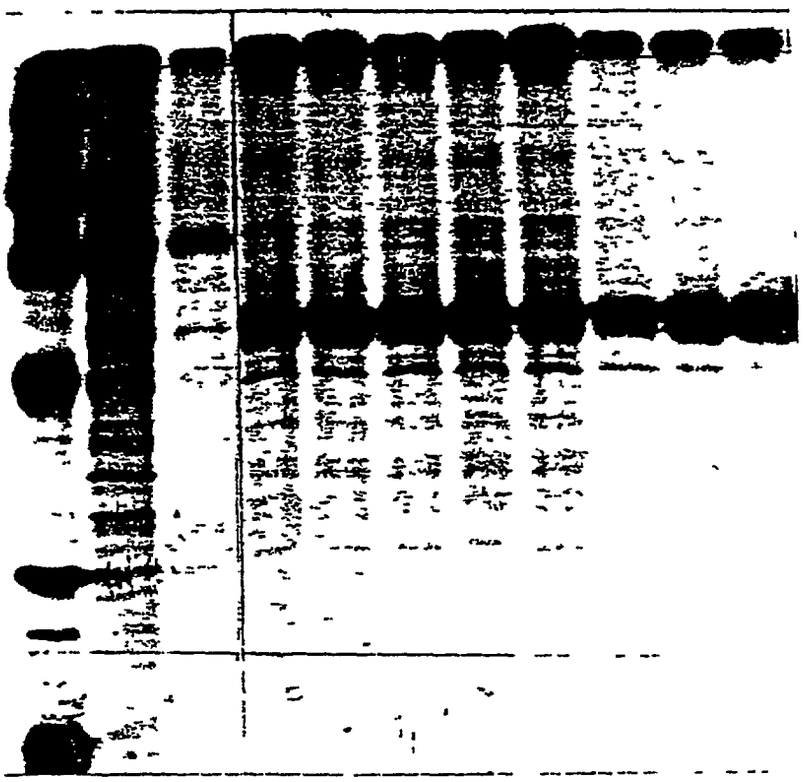

Fig.4b. SDS-PAGE analysis of the NDV polypeptides (Australia-Victoria strain) immunoprecipitated by monoclonal atibodies (lane 1-7) from lysates of virus-infected BHK-21 cells labelled with [^{35}S]-methionine. Lane (a): molecular weight markers; Lane (b) [^{35}S]-methionine-labelled virus-infected cell lysate; Lane (c). [^{3}H]-glucosamine-labelled virus-infected cell lysate. MAB clones: 1 - 3B5, 2 - 6E4, 3 - 6E6, 4 - 1F3, 5 - 1D2, 6 - 1F10, 7 - 4F11.

Fig 4 c

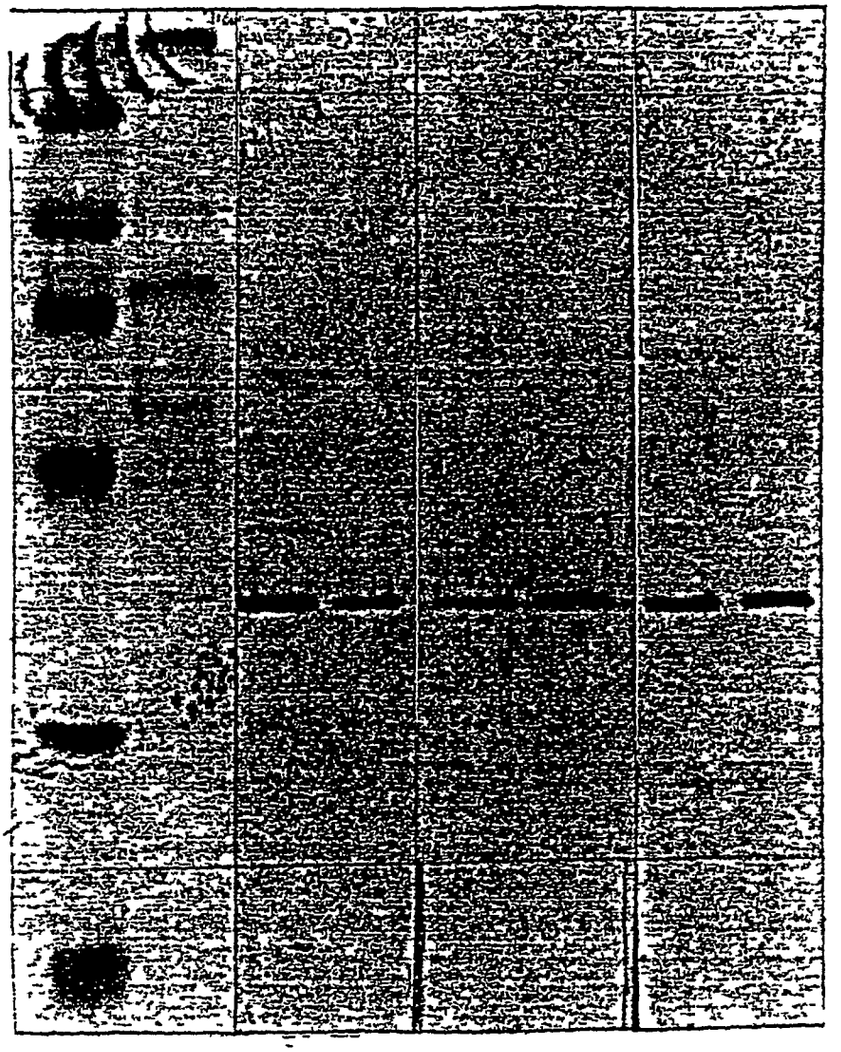

Fig. 4c SDS-PAGE analysis of the NDV polypeptides (Australia-Victoria strain) immunoprecipitated by monoclonal atibodies (lane 1-6) from lysates of virus-infected BHK-21 cells labelled with [^{35}S]-methionine. Lane (a): molecular weight markers; Lane (b) [^{35}S]-methionine-labelled virus-infected cell lysate MAB clones: 1 - 1ID9, 2 - 1BE10, 3 - 15F2, 4 - 2ID9, 5 - 4ID9, 6 - 1ID7

Fig 4d

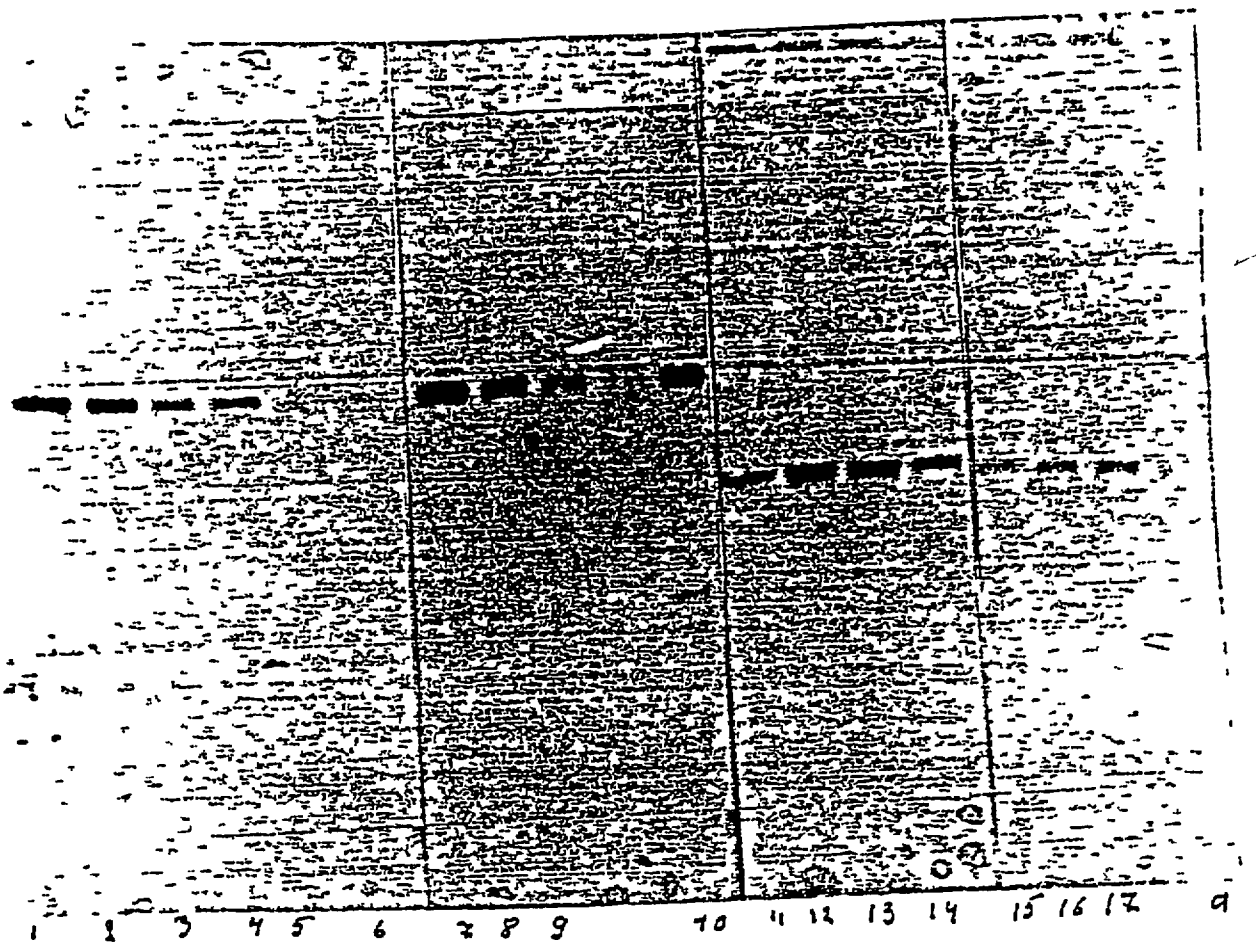

Fig 4d. SDS-PAGE analysis of the NDV polypeptides (Australia-Victoria strain) immunoprecipitated by monoclonal atibodies (lane 1-10) from lysates of virus-infected BHK-21 cells labelled with [^{3}H]-glucosamine Lane (a): [^{3}H]-glucosamine-labelled virus-infected cell lysate

MAB clones: 1 - 34C10, 2 - 42B3, 3 - 28E7, 4 - 24C8, 5 - 3E2, 6 - 14F11, 7 - 28C10, 8 - 30C2, 9 - 3E3, 10 - 2G7, 11 - 3B5, 12 - 5E4, 13 - 5E6, 14 - 1F3, 15 - 1D2, 16 - 1F10, 17 - 4F11

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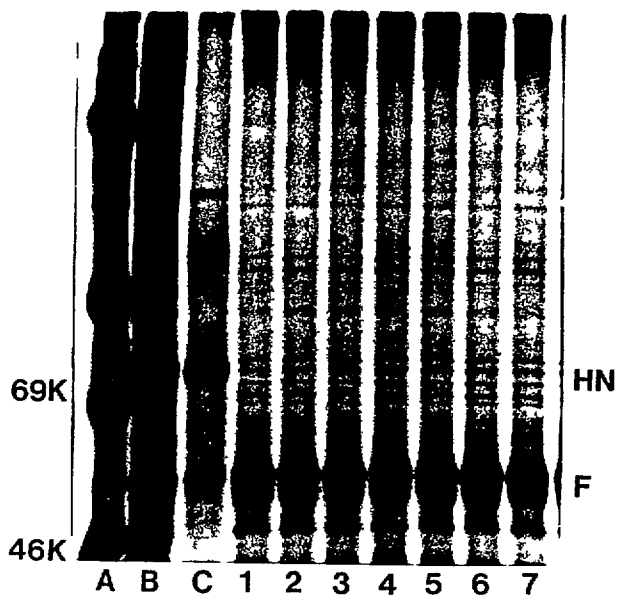
Fig 5

EPITOPE ASSIGNMENTS OF ANTI-N MONOCLONAL ANTIBODIES

Competing Antibody	Biotinylated Antibody															Antigenic site
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
1 (53d7)	+	+	+	+	+	+/-	-	-	-	-	-	-	-	+/-	-	1
2 (16g2)	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	1
3 (15c9)	+	+	+	+	+	+/-	-	-	-	-	-	-	-	-	-	1
4 (25c9)	+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	1
5 (23c10)	+	+	+	+	+	+	-	-	-	-	-	-	-	+/-	-	1 2
6 (15f10)	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	2
7 (43c3)	-	-	-	-	-	-	+	+	+	+	+	+	+	+	-	3
8 (30d4)	-	-	-	-	-	-	+	+	+	+	+	+	+	+	-	3
9 (25b11)	-	-	-	-	-	-	+	+	+	+	+,-	+ -	+	+	-	3
10 (23b1)	-	-	-	-	-	-	+/-	+	+	+	+	+	+	+ -	-	3
11 (15f3)	-	-	-	-	-	-	+/-	+	+	+	+	+	+	+/-	-	3
12 (15f2)	-	-	-	-	-	-	+/-	+	+	+	+	+	+	+	-	3
13 (15f4)	-	-	-	-	-	-	+,-	+	+	+	+	+	+	+	-	3
14 (13d7)	-	-	-	-	-	-	+	+	+	+	+	+	+	+	-	3
15 (3e11)	-	-	-	-	-	-	+ -	-	+/-	+ -	+	+	+	+	+	4

+, >60% competition, +/-, 50-60% competition, -, <50% competition

Fig 6 Immunoprecipitation assay with monoclonal antibodies against F protein of the NDV (Australia-Victoria strain)



SDS-PAGE analysis of the NDV polypeptides immunoprecipitated by monoclonal antibodies from lysates of virus-infected BHK-21 cells labelled with [35S]-methionine

Lane A - molecular weight markers

Lane B - [35S]-methionine-labelled virus-infected cell lysate

Lane C - [3H]-glucosamine-labelled virus-infected cell lysate

MAb clones 1 - F1a 2 - F1 2a 3 - F2a 4 - F2c, 5 - F2d

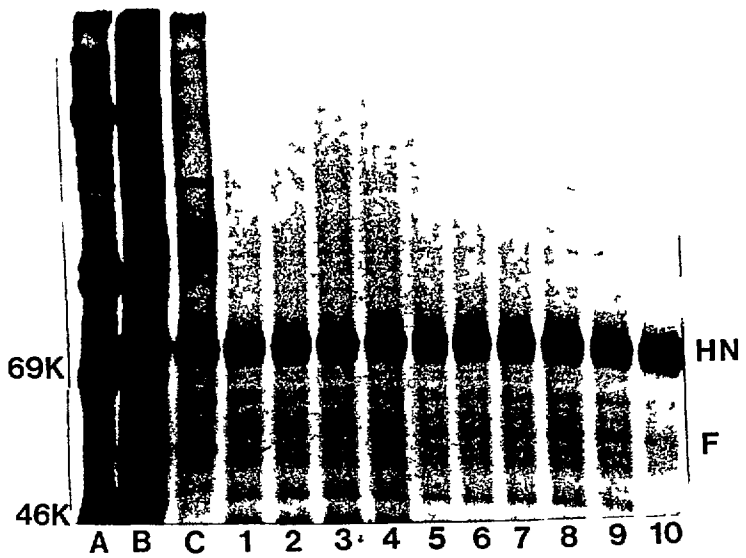
6 - F3a 7 - F3c

Fig 7 Epitope assignments of anti-HN monoclonal antibodies

Competing Antibody	Biotinylated Antibody															Antigenic Site
	HN1a	HN1b	HN1 2a	HN1 2b	HN1 2c	HN1 2d	HN1 2e	HN2a	HN2b	HN3a	HN4a	HN4b	HN4c	HN5		
HN1a	+	+	+	+	+	+	+/-	-	-	-	-	-	-	-		1
HN1b	+	+	+	+	+	+	+/-	-	-	-	-	-	-	-		1
HN1 2a	+	+	+	+	+	+	+	+	+	+/-	-	-	-	-		1 2
HN1 2b	+	+	+	+	+	+	+	+	+	-	-	-	-	-		1 2
HN1 2c	+	+	+	+	+	+	+	+	+	+/-	-	-	-	-		1 2
HN1 2d	+	+	+	+	+	+	+	+	+	+/-	-	-	-	-		1 2
HN1 2e	+	+	+	+	+	+	+	+	+	-	-	-	-	-		1 2
HN2a	-	-	+	+	+	+	+	+	+	-	-	-	-	-		2
HN2b	-	-	+	+	+	+	+	+	+	-	-	-	-	-		2
HN3a	-	-	-	-	-	-	-	-	-	+	-	-	-	-		-
HN4a	-	-	-	-	-	-	-	-	-	-	+	-	-	-		4
HN4b	-	-	-	-	-	-	-	-	-	-	+	+	+	-		4
HN4c	-	-	-	-	-	-	-	-	-	-	+	+	+	-		4
HN5a	-	-	-	-	-	-	-	-	-	-	-	-	-	+		5

+ 60% competition +- 50-60% competition, - <50% competition

Fig 8 Immunoprecipitation assay with monoclonal antibodies against HN protein of the NDV (Australia-Victoria strain)



SDS-PAGE analysis of the NDV polypeptides immunoprecipitated by monoclonal antibodies from lysates of virus-infected BHK-21 cells labelled with [35S]-methionine

Lane A - molecular weight markers

Lane B - [35S]-methionine-labelled virus-infected cell lysate

Lane C - [3H]-glucosamine-labelled virus-infected cell lysate

MAb clones 1 - HN1a 2 - HN1b 3 - HN1 2a 4 - HN1 2d 5 - HN2b

6 - HN3a 7 - HN4a 8 - HN4c 9 - HN5a 10 - HN1 2e

Fig 9 Epitope assignments of anti-HN monoclonal antibodies

Competing Antibody	Biotinylated Antibody: HN1a	HN1b	HN1 2a	HN1 2b	HN1 2c	HN1 2d	HN1 2e	HN2a	HN2b	HN3a	HN4a	HN4b	HN4c	HN5	Antigenic Site
HN1a	+	+	+	+	+	+	+/-	-	-	-	-	-	-	-	1
HN1b	+	+	+	+	+	+	+/-	-	-	-	-	-	-	-	1
HN1 2a	+	+	+	+	+	+	+	+	+	+/-	-	-	-	-	2
HN1 2b	+	+	-	+	+	+	+	+	+	-	-	-	-	-	1 2
HN1 2c	+	+	+	+	+	+	+	+	+	+/-	-	-	-	-	1 2
HN1 2d	+	+	+	+	+	+	+	+	+	+/-	-	-	-	-	1 2
HN1 2e	+	+	-	+	+	+	+	+	+	-	-	-	-	-	1 2
HN2a	-	-	+	+	+	+	+	+	+	-	-	-	-	-	2
HN2b	-	-	+	+	+	+	+	+	+	-	-	-	-	-	2
HN3a	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
HN4a	-	-	-	-	-	-	-	-	-	-	+	-	-	-	4
HN4b	-	-	-	-	-	-	-	-	-	-	+	+	+	-	4
HN4c	-	-	-	-	-	-	-	-	-	-	+	+	+	-	4
HN5a	-	-	-	-	-	-	-	-	-	-	-	-	-	+	5

+ 50% competition +- 50-60% competition, - 50% competition

Fig 10

Enders-Ruckle virus (Avian parainfluenza-2 virus)

```
AR  FASTN  (ap50.seq,  1633 bases versus viral library)
==================================================================

HNSBL 9/90: Mumps virus HANA (97.3% identity in 1294 NT overlap

 1'                                                        AACCTCAC
                                                           ::  ::·:
41" ATGATTTCTCCATCGGCAATCCACTCAACATGCCTAGCTTTATCCCAACTGCAACCTCAC

 9' CCAATGGTTGCACAAGAATTCCATCCTTTTCTTTAGGTAAGACACACTGGTGTTACACAC
    ::.:::::::·.:.::·...·. . : ::.: ..:: :::::: ::.:::::::·· :: ·
01" CCAATGGTTGCACAAGAATTCCATCCTTTTCTTTAGGTAAGACACACTGGTGTTACACAC

69' ATAATGTAATTAATGCCAACTGCAAGGATCATACTTCATCCAACCAATATGTTTCCATGG
    ::·:: :::::::: · ·... : : .·· . ·:::::::::::.:::. :::.·::·
61" ATAATGTAATTAATGCCAACTGCAAGGATCATACTTCATCCAACCAATATGTTTCCATGG

29' GGATTCTCGTTCAAACCGCGTNAGGGTATCCCATGTTCAAAACCCTAAAAATCCAATATC
    :..::·::: ::·· ·:::.  ::.   ·  ·::::::::·:·.·::.·::::::::··
21" GGATTCTCGTTCAAACCGCGTCAGGGTATCCCATGTTCAAAACCCTAAAAATCCAATATC

89' TCAGTGATGGCCTGAATCGGAAAAGCTGCTCAATTGCAANAGTCCCTGATGGTTGCGCAA
    :·.:::: : :: :   :::·. . ..·.        ::.:: : .·::·::::  ::.
81" TCAGTGATGGCCTGAATCGGAAAAGCTGCTCAATTGCAACAGTCCCTGATGGTTGCGCAA

49' TGTACTGTTACGTTTCAACTCAACTTGAAACCAACGACTATGCGGGGTCCAGCCCACCTA
    :::·:·::.:.:: :   ··.  ::::::.:::::·.· ::::::: ::::  :::· ::
41" TGTACTGTTACGTTTCAACTCAACTTGAAGCCAACGACTATGCGGGGTCCAGCCCACCTA

09' CCCAGAAACTTACCCTGTTATTCTATAATGACACCATCACAGAAAGGACAATATCTCCAT
     ::.::·:·: ·::::     ·:·:::::::::::::::. ·:::::::. ::: ·::::. ::
01" CCCAGAAACTTACCCTGTTATTCTATAATGACACCATCACAGAAAGGACAATATCTCCAT

69' CTGGTCTGGAAGGGAATTGGGCCACTTGGGTGCCAGGAGTGGGGAGGGGAATATATTTCG
    ··:· : ·::: : ::· .  :: :  ·::.:::::::·   :· ::· .::. .::  ::
61" CTGGTCTTGAAGGGAATTGGGCTACTTTGGTGCCAGGAGTGGGGAGTGGAATATATTTCG

29' AAAATAAGTTGATCTTTCCTGCATATGGGGGTGTCTTGCCCGATAGTACACTAGGAGTTA
      :: :: :·:·:: ::.:·::            : :.: :: :::  ::: ·:::
21" AAAATAAGTTGATCTTTCCTGCATATGGGGGTGTCTTGCCCAATAGTACACTAGGAGTTA

89' AATCAGCAAGAGAATTTTTCCGGCCCGTTAATCCATATAATCCATGTTCAGGACYACCAC
    : ·:· : ::.: :·   :::   .:::         ::::: :. ·.:: ·::.· ::·
81" AATCAGCAAGAGAATTTTTCCGGCCCGTTAATCCATATAATCCATGTTCAGGACCACCAC

49' AAGAGTTAGATCAGCGGGCTTGGAGATCATATTTCCCAAGATACTTCTCTAGTCGAAGGG
    ::: :· : : .: :.    ::·:·· :·::·::::.  .·:·::·. ::·   ::  ::
41" AAGAGTTAGATCAGCGTGCTTTGAGATCATATTTCCCAAGATACTTCTCTAGTCGAAGGG

09' TACAGAGTGCATTTCTGGTCTGTGCCTGGAATCAGATCCTAGTTACAAATTGCGAGCTAG
    : ·:.:: : : ·· :::: ··             :::: .    ·::  :::·..::
01" TACAGAGTGCATTTCTGGTCTGTGCCTGGAATCAGATCCTAGTTACAAATTGCGAGCTAG

69' TTGTCCCCTCAAACAATCAGACACTGATGGGTGCAGAAGGAAGAGTTTTATTGATCAATA
    :: :::: :: : :·  ::·      :·:  ·:·  ·::::::   :::.  :::· :.
61" TTGTCCCCTCAAACAATCAGACACTGATGGGTGCAGAAGGAAGAGTTTTATTGATCAATA
```

118

Fig 10
continuation

```
      :: :.:: :::.:.:::.:.. .:::::::::::.: ..:::::.::::.::::::.::::
   21" ATCGGCTATTATATTATCAGAGGAGTACTAGCTGGTGGCCGTATGAACTCCTCTATGAGA

  789' TATCATTCACATTTACAAACTCTGGTCAATCATCTGTGAATATGTCCTGGATACCTATAT
       :::.::.::.:::::::.. ..:.:::::.::::.:.:::::::::::::.:::::::
 1381" TATCATTCACATTTACAAACTCTGGTCAATCATCTGTGAATATGTCCTGGATACCTATAT

  849' ATTCATTCACTCGTCCTGGTTCGGGCAACTGCAGTGGTAAAAATGTATGCCCANCAGTCT
       :: :: : ::.:::.:.::::.:.:.:.  .:::::::: .::::.::: :.:::::.
 1441" ATTCATTCACTCCTCCCGGTTCGGGCAACTGCAGTGGTAAAAATGTATGCCCAACAGTCT

  909' GTGTATCAGGAGYTTATCTTGATCCCTGGCCATTGACTCNATACNGCNACYAATNAGGCA
       ::.:.::::.:. ..:::. . .::.:::: ::.:.:.  ..:::::.::.:...:...:::::
 1501" GTGTATCAGGAGTTTATCTTGATCCCTGGCCATTAACTCCATACAGCCACCAATCAGGCA

  969' TTAACAGAAATTTCTATTTCACAGGTGCACTGCTAAATTCAAGCACAACNAGGGTGAATC
       ::::::::::::.:: .:::::.     .::::::::::: .:::::::.::.::::::: ::
 1561" TTAACAGAAATTTCTATTTCACAGGTGCACTGCTAAATTCAAGCACAACCAGGGTGAATC

 1029' CTACCCTTTATGTCTCTGCCCTTAATAATCTTAAAGTTCTAGCCCCATATGGTACTCAAG
       ::: ::::::.::.:::::::::::: :. ..:::.::::::::::::: :::::::
 1621" CTACCCTTTATGTCTCTGCCCTTAATAATCTTAAAGTTCTAGCCCCATATGGTACTCAAG

 1089' GATTGTTTGCATCATACACCACAACNACCTGCTTTCAAGATACCGGTGACGCTAG-GTGT
       ::: .:::::.::.:::..:::::::..:: ..:  :  .::::::::::::::: ::::::
 1681" GATTGTTTGCATCATACACCACAACCACCTGCTTTCAAGATACCGGTGACGCTAGTGTGT

 1148' ATTGTGTCTATATTATGGAACNAGCATCGAATATTGTTGGAGAATTCCAAATTCTACCTG
       ::::.::::::.::: :::..   ::.:::::::::::::  ::: :::: :::.:::.::
 1741" ATTGTGTCTATATTATGGAACTAGCATCGAATATTGTTGGAGAATTCCAAATTCTACCTG

 1208' TGCTAGCCAGATNNNCCATCACTTGAGTNGTAGTGAAYGNAGCAGGAAGCTTTATGGGCG
        .:: ::::: .. .:::::. :     .: ::::::.:. .:::: :::  ::  ::
 1801" TGCTAGCCAGATTGACCATCACTTGAGTTGTAGTGAATGTAGCAGGAAGCTTTATGGGCG

 1268' TGTCTCATTTCTTATCGATTATTAAGAANNNNNNGGCCAGAANNGGCCTAAATGAGATAT
       : .:: ::::: ::: .:::[redacted]  Start of L gene
 1861" TGTCTCATTTCTTATCGATTATTAAG  Poly-A
                    TATA Box
```

MUMMPSHN 3/90, Mumps virus HN mRNA for hemagglutinin, 92.3% identity

```
    1'                                                        AACCTCA
                                                              :::::::
  541" CATGATTTCTCTATCGGCCATCCACTCAACATGCCTAGCTTTATCCCAACTGCAACTTCA

    8' CCCAATGGTTGCACAAGAATTCCATCCTTTTCTTTAGGTAAGACACACTGGTGTTACACA
       ..:: ::: .::::::.:::::: . ::  ...:::  ..:::::::::::::.:::::::
  601" CCCAATGGTTGCACAAGAATTCCATCCTTTTCTCTAGGTAAGACACACTGGTGCTACACA
```

Fig 10

Continuation

```
   76' CTCCTCTANGAGATATCATTCACATTTACAAACTCTGGTCAATCATCTGTGAATATGTCC
       ::.::::::.: :'':.:: ::::         ::::::::::.'::'::: : ::. :: .:
  381" CTCCTCTATGAGATATCATTCACATTTACAAACTCTGGTCAATCATCTGTGAATATGTCC

  836' TGGATACCTATATATTCATTCACTCGTCCTGGTTCGGGCAACTGCAGTGGTAAAAATGTA
       :'::'::::'::.::::::' ::'.    .'    '::[illegible]:' ::[illegible]::.::::'::[illegible]::::.:::
 1441" TGGATACCTATATATTCATTCACTCGTCCTGGTTCAGGCAAATGCAGTGGTGAAAATGTA

  896' TGCCCANCAGTCTGTGTATCAGGAGYTTATCTTGATCCCTGGCCATTGACTCNATACNGC
       ::::::.[illegible]:[illegible]'::.::[illegible] ::  [illegible]    '.::::::::::  :::::[illegible].:[illegible]:.' :[illegible].'::
 1501" TGCCCAATTGCTTGTGTGTCAGGGGTTTATCTTGATCCCTGGCCATTAACGCCATATAGC

  956' NACYAATNAGGCATTAACAGAAATTTCTATTTCACAGGTGCACTGCTAAATTCAAGCACA
       .::.'::..:.: ::' ::.'::::'::::::::'' :''::::::'.:[illegible]::::::.::..::
 1561" CACCAATCAGGCATTAACAGAAATTTCTATTTCACAGGTGCACTATTAAATTCAAGCACA

 1016' ACNAGGGTGAATCCTACCCTTTATGTCTCTGCCCTTAATAATCTTAAAGTTCTAGCCCCA
       : .'::[illegible]::.:'''' '::: [illegible]          .'::::::.'::[illegible]::::::' :[illegible]::.'::.::
  621" ACCAGAGTAAATCCTACCCTTTATGTCTCTGCCCTTAATAACCTTAAAGTACTAGCCCCA

  076' TATGGTACTCAAGGATTGTTTGCATCATACACCACAACNACCTGCTTTCAAGATACCGGT
       :::::: ::::'::'[illegible]::.[illegible]:: [illegible]::[illegible]:::.'::''.::.::  :: :::::.::.'':.
 1681" TATGGTACTCAAGGACTGTCTGCCTCGTACACCACAACCACCTGCTTTCAAGATACCGGT

 1136' GACGCTAG-GTGTATTGTGTCTATATTATGGAACNAGCATCGAATATTGTTGGAGAATTC
       ::[illegible]::[illegible].:[illegible]'': : : ::: [illegible]'': ::::::       : .::..::::[illegible].:::'::::'::.
 1741" GATGCCAGTGTGTATTGTGTTTATATTATGGAACTAGCATCGAATATCGTTGGAGAATTC

  195' CAAATTCTACCTGTGCTAGCCAGATNNNCCATCACTTGAGTNGTAGTGAAYGNAGCAGGA
       :'.::::::..: :'::'::[illegible]::        [illegible]'::::::::::'.[illegible]: ::'.  :.  :[illegible].:
  801" CAAATTCTACCTGTGCTAACCAGATTGACTATCACTTGAGTAATAGTGAATGCAGCGAGA

 1255' AGCTTTATGGGCGTGTCTCATTTCTTATCGATTATTAAGAANNNNNNGGCCAGAANNGGC
       [illegible]: .[illegible]:'[illegible].'': :.' '::. [illegible]:'[illegible].'': :'  .:[illegible]
 1861" GGNNCTACNGGNGTGTCTCAATTTTTATCGATTATTAAGA
```

Poly-A

Start of L gene

TATA-Box

Fig 11

Enders-Ruckle virus (Avian parainfluenza-2 virus)

SAR FASTN (ap50 seq, 1633 bases versus viral library)

MUMHN Mumps virus hemagglutinin-neuraminidase, 91.8% identity

```
   1'      AACCTCACCCAATGGTTGCACAAGAATTCCATCCTTTTCTTTAGGTAAGACACAC
           :. ¶· :: ·:      .      .       :::::: :¶     ·.·    ::
 601" ACTGCAACTTCACCCAATGGTTGCACAAGAATTCCATCCTTTTCTCTAGGTAAGACACAC

  56' TGGTGTTACACACATAATGTAATTAATGCCAACTGCAAGGATCATACTTCATCCAACCAA
      ::  .¶ :¶ ·:· ·   .·¶:.    .:: ·· . :::::.        .¶      :::
 661" TGGTGCTATACACATAATGTGATTAATGCCAACTGCAAGGATCATACTTCGTCCAACCAA

 116' TATGTTTCCATGGGGATTCTCGTTCAAACCGCGTNAGGGTATCCCATGTTCAAAACCCTA
      :: .:: :: ·:. :   .:..:. ¶::::.:. :::::::·  ¶ .:        .¶:·
 721" TATGTTTCCATGGGGATTCTCGTTCAGACCGCGTCAGGGTATCCTATGTTCAAAACCTTA

 176' AAAATCCAATATCTCAGTGATGGCCTGAATCGGAAAAGCTGCTCAATTGCAANAGTCCCT
      :: .:· :  :: ::   : ..::.·::·:::::::::::·   .·:::::::.:·::::·
 781" AAAATCCAATATCTCAGTGATGGCCTGAATCGGAAAAGCTGCTCAATTGCAACAGTCCCT

 236' GATGGTTGCGCAATGTACTGTTACGTTTCAACTCAACTTGAAACCAACGACTATGCGGGG
      : ·::¶:: ::  :   ::::..:::¶:.·::::::::   ¶..:¶:::·::::·::.
 841" GATGGATGCGCAATGTACTGTTACGTCTCAACTCAACTTGAGACCGACGACTATGCGGGG

 296' TCCAGCCCACCTACCCAGAAACTTACCCTGTTATTCTATAATGACACCATCACAGAAAGG
        .. ·· ::  :: ::::·    ¶.::.   .:.:¶: · · ::::: ¶  .
 901" TCCAGCCCACCTACCCAGAAACTAACCCTGTTATTTTATAATGACACCGTCACAGAAAGG

 356' ACAATATCTCCATCTGGTCTGGAAGGGAATTGGGCCACTTGGGTGCCAGGAGTGGGGAGG
       .:  :: ·:  :  ..          .      . ¶    ¶·::.:             ¶
 961" ACAATATCTCCATCTGGTCTGGAAGGGAATTGGGCTACTTTGGTGCCAGGAGTGGGGAGT

 416' GGAATATATTTCGAAAATAAGTTGATCTTTCCTGCATATGGGGGTGTCTTGCCCGATAGT
       :¶  :· .·  : ¶:·   ¶    .·                .:: :        ¶
1021" GGTATATATTTCGAGAATAAATTGATCTTTCCTGCATATGGGGGTGTCTTGCCCAATAGT

 476' ACACTAGGAGTTAAATCAGCAAGAGAATTTTTCCGGCCCGTTAATCCATATAATCCATGT
       .:  ¶: .:     :¶.                .. ¶  ¶   :·::        ¶    ¶
1081" ACACTCGGAGTTAAATTAGCAAGAGAATTTTTCCGACCTGTTAATCCATATAACCCATGC

 536' TCAGGACYACCACAAGAGTTAGATCAGCGGGCTTGGAGATCATATTTCCCAAGATACTTC
       ·..  ·  :¶ :: .·¶      ·::: ¶..::¶:.      ·¶:       ¶    ¶
1141" TCAGGACCACAACAAGATTTAGATCAGCGTGCTTTGAGATCATACTTCCCAAGTTACTTA

 596' TCTAGTCGAAGGGTACAGAGTGCATTTCTGGTCTGTGCCTGGAATCAGATCCTAGTTACA
         :¶ ·. : ¶:  ···     :. :::¶:  :..:.·     ::
1201" TCTAATCGAAGAGTACAGAGTGCATTTCTTGTCTGTGCCTGGAATCAGATCCTAGTTACA

 656' AATTGCGAGCTAGTTGTCCCCTCAAACAATCAGACACTGATGGGTGCAGAAGGAAGAGTT
        :.  : ..   : ·· ·· ·::::·····:: :::::::       .:
1261" AATTGCGAGCTAGTTGTCCCCTCAAACAATCAGACACTGATGGGTGCAGAAGGAAGAGTT

 716' TTATTGATCAATAATCGGCTATTATATTATCAGAGGAGTACTAGCTNGCNGCCGTATGAA
       ::   :  .  .: .:¶.·:...:·.:·::::::·¶:::: ¶   :.·¶
1321" TTATTGATCAATAATCGACTATTATATTATCAGAGAAGTACCAGCTGGTGGCCGTATGAA
```


121

Fig 11

Continuation

```
  76' CTCCTCTANGAGATATCATTCACATTTACAAACTCTGGTCAATCATCTGTGAATATGTCC
 381" CTCCTCTATGAGATATCATTCACATTTACAAACTCTGGTCAATCATCTGTGAATATGTCC

 836' TGGATACCTATATATTCATTCACTCGTCCTGGTTCGGGCAACTGCAGTGGTAAAAATGTA
1441" TGGATACCTATATATTCATTCACTCGTCCTGGTTCAGGCAAATGCAGTGGTGAAAATGTA

 896' TGCCCANCAGTCTGTGTATCAGGAGYTTATCTTGATCCCTGGCCATTGACTCNATACNGC
1501" TGCCCAATTGCTTGTGTGTCAGGGGTTTATCTTGATCCCTGGCCATTAACGCCATATAGC

 956' NACYAATNAGGCATTAACAGAAATTTCTATTTCACAGGTGCACTGCTAAATTCAAGCACA
1561" CACCAATCAGGCATTAACAGAAATTTCTATTTCACAGGTGCACTATTAAATTCAAGCACA

1016' ACNAGGGTGAATCCTACCCTTTATGTCTCTGCCCTTAATAATCTTAAAGTTCTAGCCCCA
1621" ACCAGAGTAAATCCTACCCTTTATGTCTCTGCCCTTAATAACCTTAAAGTACTAGCCCCA

1076' TATGGTACTCAAGGATTGTTTGCATCATACACCACAACNACCTGCTTTCAAGATACCGGT
1681" TATGGTACTCAAGGACTGTCTGCCTCGTACACCACAACCACCTGCTTTCAAGATACCGGT

1136' GACGCTAG-GTGTATTGTGTCTATATTATGGAACNAGCATCGAATATTGTTGGAGAATTC
1741" GATGCCAGTGTGTATTGTGTTTATATTATGGAACTAGCATCGAATATCGTTGGAGAATTC

1195' CAAATTCTACCTGTGCTAGCCAGATNNNCCATCACTTGAGTNGTAGTGAAYGNAGCAGGA
1801" CAAATTCTACCTGTGCTAACCAGATTGACTATCACTTGAGTAATAGTGAATGCAGCGAGA

1255' AGCTTTATGGGCGTGTCTCATTTCTTATCGATTATTAAGAANNNNNNGGCCAGAANNGGC
1861" GGNNCTACNGGNGTGTCTCAATTTTTATCGATTATTAAGA
```

Poly-A *Start of L gene*

TATA-box

Fig 12

ENDERS-RUCKLE virus (ERV) - Mumps virus (MuV) alignment

L Gene

95 9% identity in 340 bases overlapping

```
1 - 70      nGGCCAGAAnnGGGCCCCTAAATGAGATATTCCTACCCGAAGTACATTTAAACnnCCCCATCGTTAGATA  ERV
            !!!!!!!!!  ! ! !!!!!!!!!!!!!!! !! !!!! !! !!!!!!!!!!!!   !!!!!!!!!!!!!!
            AGGCCAGAATGGCGGGCCTAAATGAGATACTCTTACCTGAGGTACATTTAAACTCACCCATCGTTAGATA  MuV

71 - 140    TAAGCTTTTCTACTATATATTGCATGGCCAGTTACCAAAnGATTTGGAGCCAGATGACTTGGGCCCATTA  ERV
            !!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!! !!!!!!!!!!!!!!!!!!!!!!!!!!!!!!
            TAAGCTTTTCTACTATATATTGCATGGCCAGTTACCAAATGATTTGGAGCCAGATGACTTGGGCCCACTA  MuV

141 - 210   GCAAATnAGAATTGGAAGGCAATTCGAGCTGAAGAATCCnAGGTTCATGCACGGTTAAAACAGATCAGAG  ERV
            !!!!!! !!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!! !!!!!!!!!!!!! !!!!!!!!!!!!!!!!!
            GCAAATCAGAATTGGAAGGCAATTCGAGCTGAAGAATCCCAGGTTCATGCACGTTTAAAACAGATCAGAG  MuV

211 - 280   nAGAACTCATTGCAAGGATTCCTGGTCTCCGGTGGACCCGCTCTCAAAGAGATATTGCCATACTCATTTG  ERV
             !!!!!!!!!!!!!!!!!!!!!!! !!!!!!!!!!!!!!!!!!!!!!!! !! !! !!!!!!!!!!!!!!!!!
            TAGAACTCATTGCAAGGATTCCTAGTCTCCGGTGGACCCGCTCTCAGAGGGAGATTGCCATACTCATTTG  MuV

281 - 340   GCCAAGAATnCTTCCAATACTGCAAGCATATGATCTTCGGCAAAGTATGCAATTn  ERV
            !!!!!!!!! !!!!!!!! !!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!!
            GCCAAGAATACTTCCAATCCTGCAAGCATATGATCTTCGGCAAAGTATGCAATTG  MuV
```

Fig 13

HUSAR FASTN

LIGNMENT: MUMNPLGZ. Mumps virus nucleocapsid (NP) mRNA
7.7% identity in 544 bases overlapping

```
==========================================================================

   1'                                                GCNGGGANACNAGGNGGG
                                                     ':.:::: .:.::'.::
1321" GAGGTTACTCAGCTTATTGACAGGCTTGCAAGGGGCAGAGGTGCGGGGATACCAGGTGGG

  19' CCTGTGAATCCTTTNGTTCCTCCAGTTCAACAGCAACAACCTGCTGCCGTATATGCGGAC
      .: ::':::::::::.:.:'.:'   ::::::::::.:::'::::'.:.:: :.::: :'::
1381" CCCGTGAATCCTTTTGTTCCTCCAGTTCAACAGCAACAACCTGCTGCCGCATATGAGGAC

  79' ATCCCNGCATNGGATGAATCAGATGACAATGGTGATGAAGATGGAGGCGCAGGATTCCAA
      .: ':.:' :.::: :  :  ::' .  : ::' ::'::'::: :: :::'' :::: :
1441" ATTCCTGCATTGGAGGAATCAGATGACGACGGTGATGAAGATGGGGGTGCAGGATTCCAA

 139' AATGGAGNACAAGYACNAGCTATAAGANNNGNAGGTCAAACTGACTTTAGAGCACAGCCT
      :::: : .':.: .::.: :'  :::     .::. ..:: '.:. : . :: : .:::
1501" AATGGAGCACAAGCACCAGCTGCAAGACAGGGAGGCCAAAATGACTTCAGAGTACAGCCA

 199' TTACAAGATCCAATTCAAGCACAACTTTTCATGCCATTATATCCTCAAGTCAGCAACATC
       :.:: : :.:':.::.:: .:. ..::::'.::'::: .:::.::.:::  ::::  .:'
1561" CTACAGGATCCAATTCAAGCACAACTTTTCATGCCATTATATCCTCAAGTCAGCAACATC

 259' CCAAATAATAAGAATNATCATATCAATCGCATNNNNNGGCTGGAAAACCAAGATTNATNA
        '' : :   : :'.:  :  ':::     :. . .:: .:: ' ' ::: . :.:' :
1621" CCAAATCATCAGAATCATCAGATCAATCGCATCGGGGGGATGGAACACCAAGATTTATTA

 319' CGATACAACGAGAATGGNGATTCTCAACAAGANGCAANNNGCGAACACGGAAACACTTTC
      .: :: . : : : :' .: ::    ::::' .::::....: ::  :: .::::.   :'
1681" CGATACAACGAGAATGGTGATTCTCAACAAGATGCAAGGGGCGAACACGGAAACACCTTC

 379' CCAAACNNNNCCAATCAAAACGCACAGTTGCAAGTGGGCGACTGGGATGAGTAAATCACT
        :':' . .  '' :: ::'  :::    .:  :::'  ::'':: ::   .:::  ::
1741" CCAAACAATCCTAATCAAAACGCACAGTCGCAAGTGGGCGACTGGGATGAGTAAATCACT

 439' GACATGATCAAACTAACCTCAATTGCAAGAATCCTAGGACAATCNANGCCACAGCTAACT
      :: : .:'' : : :  :  .:'  '':: :: .:   '':: '':: '   :::'   ::'
1801" GACATGATCAAACTACCCCCAATTGCAATAACCCCAGGACAATCTA-GCCACAGCTAACT

 499' GCCCAAATCCACNACACTCCATTCATATTNAGTCTT TAAG AAAAAA CTAGGCCCGGAAAG
        ' :: : : : ::: ' ::  .':'    :: :: [illegible] ::::::
1860" GCCCAAATCCACTACATTCCATTCATATTTAGTCTT TAAG AAAAAA
                                      -=<=----------   ---------=>=-
                                        TATA Box  Poly-A

                        Putative END of the NP Gene    START  P Gene
```

Fig 14

Avian mumps-like virus (AmuV)

HUSAR FASTN

ALIGNMENT· MUMMP 12/89· Mumps virus, Miyahara strain, P gene
90.8% identity in 87 bases overlapping

```
==================================================================

 421' TGGGATGAGTAAATCACTGACATGATCAAACTAACCTCAATTGCAAGAATCCTAGGACAA  AMuV
                                          ::::::::: :: :: ::.::.:
   1"                                    CCAATTGCAATAACCCCAGGACAA  Mumps

 481' TCNANGCCACAGCTAACTGCCCAAATCCACNACACTCCATTCATATTNAGTCTTTAAGAA  AMuV
      .:.· : :::·::·::.:::::::: .. .::: ..:::·::·::.::.::::::::::
  25" TCTA-GCCACAGCTAACTGCCCAAATCCACTACATTCCATTCATATTTAGTCTTTAAGAA  Mumps
                                                     -=<=----------
 -=<=------   intergenic region                Putative END of the NP Gene
 541' AAAA
      ::::   /   concensus ?
  84" AAAA CT AGGCCCGGAAAGAA   TTAGTTCTACGAGCATCGACACAATTATCTTGATCGTGTT
              ----------------=>=-
            Putative START of the P Gene                Mumps virus
```

Fig 15 Sequence of the portion of the APMV-1/Show pigeon/Israel/1831/84 virus obtained by annealing with a primer to the F gene of the NDV

AGnAACTCAC ACAATCnCCT CCannTTCTC TGGCTnAGAA CAGAATnaAG cAGCTGAGAT

GTTGTGTTTG TTGCGTTTGT GGGGTCACnA CTTnAGtCCG TAGTGCAGCA AncGcAGCCA

GGAGCCAGAT GtGCGCnCCn aAGATGGtAG ACTTCGAtAT GATCCTCCaA GyATnATCTT

TCTTtnnGGG aACaATnATC aATGGAtACA GGaAGnAGaA TTnAGGTGTG TGGCCACnAG

tcaaGAtAGA yACGAyAyAT gGGcaGGTCA TTgGGCAGCn ACATGCTGCT CAAGCAGaGA

TTTCACATGA TGTCATGTTG AGnGAGTaCa AGAGTTnATC TGCCCTtGAA TTtGAGCCAT

GTATAGAATA TGACCCtgTC ACCAATCtAA GTATGTTTTT AAAGGACAAG GCAAtnGCAC

ATCCGAAAGA TAACTGGCTC GCCTCATTnA GGCGGAACCT TCTCTCTGAG GACnAGAAGA

AACAGGTAAA AgaGGCAACC TCAACTAACc GCCTCTtGAT AGAGTTCTTA GAATCAAAtG

ATTTTGATCC AtAtAAGGAG AtGGAAtACT nGACAACCCT tGAGtACCTA AGAGACGACA

GTGTGGCGGT ATCGTATTCA CTCAAGGAGA AGGAGGTGAA GGTtAATngG CGGATTTTCG

CTAAGTTGAC AAAGAAGTTA AGGAACtGCc aGGtAATGsA AcAcGGGATT CTAGCTGACC

AGATTGCACC TTTTTTCCAG GGAAATGGGG TCATTCAAGA TAGCATATCC TTGACTAAGA

GTAtGTTAGC GATGAGTCAA CTGTCTTTTA ACAGCAAtAA GAAACGTATC ACTGACTGCA

AGGAGAGGGT TTCCTCAAAC CGCAATCATG ATCCGAAGAG CAAGAATCGT CGAAGAGTCG

CCaCTTTTAT CACGACTGAC CTGCAAAAGt ATTGTCTTAA CTGGAGATAT

Fig 16 Alignment between the APMV-1/Show pigeon/Israel/1831/84 (APMV-1/P) and the L gene of NDV

(FASTN program HUSAR, 84 0% identity in 954 NT overlap)

```
   1'                                  AGNAACTCACACAATCNCCTCCANNTT  APMV-1/P
1021  AATCGGCCTCCTCCCCAATGATATAGCAGAATCCGTGACTCATGCAATAGCTACTGTATT  NDV
  28' CTCTGGCTNAGAACAGAATNAAGCAGCTGAGATGTTGTGTTTGTTGCGTTTGTGGGGT--
1081  CTCTGGTTTAGAACAGAATCAAGCAGCTGAGATGTTGTGCCTGTTGCGTCTGTGGGGTCA
  86' --CACNACTTNAGT-CCGTAGTGCAGCAANCGCAGCCAGGAGCCAGATGTGCGCNCCNAA
1141  CCCACTGCTTGAGTCCCGTATTGCAGCAAAGGCAGTCAGGAGCCAAATGTGCGCACCGAA
 143' GATGGTAGACTTCGATATGATCCTCCAAGYATNATCTTTCTTTNNGGGAACAATNATCAA
1201  AATGGTGGACTTTGATATGATCCTTCAGGTACTGTCTTTCTTCAAGGGAACAATCATCAA
 203' TGGATACAGGAAGNAGAATTNAGGTGTGTGGCCACNAGTCAAGATAGAYACGAYAYATGG
1261  CGGATACAGAAAGAAGAATGCAGGTGTGTGGCCGCGAGTCAAAGTGGATACAATATATGG
 263' CCAGGTCATTGGGCAGCNACATGCTGCTCAAGCAGAGATTTCACATGATGTCATGTTGAG
1321  GAAGATCATTGGGCAACTACATGCAGATTCAGCAGAGATTTCACACGATATCATGTTGAG
 323  NGAGTACAAGAGTTNATCTGCCCTTGAATTTGAGCCATGTATAGAATATGACCCTGTCAC
1381  AGAGTATAAGAGTTTATCTGCACTTGAATTTGAGCCATGTATAGAATACGACCCTGTCAC
 383  CAATCTAAGTATGTTTTTAAAGGACAAGGCAATNGCACATCCGAAAGATAACTGGCTCGC
1441  TAACCTGAGCATGTTCCTAAAAGACAAGGCAATCGCACACCCTAACGATAATTGGCTTGC
 443' CTCATTNAGGCGGAACCTTCTCTCTGAGGACNAGAAGAAACAGGTAAAAGAGGCAACCTC
1501  CTCGTTTAGGCGGAACCTTCTCTCCGAAGACCAGAAGAAACATGTAAAAGAAGCAACTTC
 503' AACTAACCGCCTCTTGATAGAGTTCTTAGAATCAAATGATTTTGATCCATATAAGGAGAT
1561  GACTAATCGCCTCTTGATAGAGTTTTTAGAGTCAAATGATTTTGATCCATATAAAGAGAT
 563' GGAATACTNGACAACCCTTGAGTACCTAAGAGACGACAGTGTGGCGGTATCGTATTCACT
1621  GGAATATCTGACGACCCTTGAGTACCTTAGAGATGACGATGTGGCAGTATCATACTCGCT
 623' CAAGGAGAAGGAGGTGAAGGTTAATNGGCGGATTTTCGCTAAGTTGACAAAGAAGTTAAG
1681  CAAAGAGAAGGAAGTGAAAGTTAATGGACGGATCTTCGCTAAGCTGACAAAGAAGTTAAG
```

Fig 16 Continuation

683' GAACTGCCAGGTAATGNAACACGGGATTCTAGCTGACCAGATTGCACCTTTTTTCCAGGG APMV-1/P

1741 GAACTGTCAGGTGATGGCGGAAGGGATCCTAGCCGACCAGATTGCACCTTTCTTTCAGGG NDV

743' AAATGGGGTCATTCAAGATAGCATATCCTTGACTAAGAGTATGTTAGCGATGAGTCAACT

1801 AAATGGAGTCATTCAGGATAGCATATCGTTGACCAAGAGTACGCTAGCGATGAGTCAACT

803' GTCTTTTAACAGCAATAAGAAACGTATCACTGACTGCAAGGAGAGGGTTTCCTCAAACCG

1861 GTCTTTTAACAGCAATAAGAAACGTATCACTGACTGTAAAGAAAGAGTATCTTCAAACCG

863' CAATCATGATCCGAAGAGCAAGAATCGTCGAAGAGTCGCCACTTTTATCACGACTGACCT

1921 CAATCATGATCCCAAGAGCAAGAACCGTCGGAGAGTTGCAACCTTCATAACAACTGACCT

923' GCAAAAGTATTGTCTTAACTGGAGATAT

1981 GCAAAAGTACTGTCTTAATTGGAGATATCAGACAATCAAACTGTTCGCTCATGCCATCAA

Fig 17

Sequence of the genome portion PMV-1/Racing pigeon/Israel/1831/84
753 nucleotides

ACtACTGTTA TACTCACAAC GtATTATTGT CTGGCTGCAG AGATnACTcA CACTCACATc
AAtACTnAGC ACTTGGTGTG CTTCGGACAT CTGCAAcaGG naGGGtATTC TTTTCtACTC
TGCGTTCCAT CAATTnAGAT GACACCCAAA ATCGGAAGTC TTGCAGTGTG AGTGcAACTC
CTCTAGGTTG TGAtATACTG TGCTCCAaAG TCACAGAGAC TGAAGAAGAG GATtATAagt
nAGTnnCCCC CACATCAATn GTGCACGGAA GGTtAGGTTT tgACGGTCAA TACCATGAGA
AGGACTtAGA CATTACAGTC TTATTTAAGG ATtGGGTGGC AAATnnCCCG CCACnGGGAn
gCGGATCTTT yAttAAygAC CGyGyAyGGT TCCCAGTTyA CGGaGGGCTA AAACCTAATT
CGCCACTAGC CaCTGCACAA GaaGGGAAAt AyGTAAyATA CaAGCGCTAT AATAACACAT
nCCCCGATGG ACAAGAAtAT CAAATCCGGA TGGCTAAGTC TtCGTACAAG CCtGGGCATT
CGGTGGAAAG CGTGTACAGC AAGCCATCTT ATCtATCAAA GtGTCAACAT CTTTAGGTGA
GGACCCGGTG TTGACTGTTC CGCCTAATAC AATTACACTC ATGGGGGCCG AcgGAGTTCT
CACAGTAGGG ACATCTCATT TCTTGTACCA ACGAGGGTCT TCGTATTTCT CCCCTGCCTT
ATTATATCCT ATGACAGTCT ATAACAAAAC Gnn

Fig 18

Alignment of the sequences portion of the APMV-1/show pigeon/1831/84 portion with the HN gene of the Newcatle disease virus, 89 5% identity in 754 nucleotide overlapping

```
   1                                                ACTACTGTTATACTCACAA      APMV-1 isolate
 601  CGGTTGCACTCGGATACCCTCATTTGACATGAGCACCACTCACTACTGTTATACTCACAA      NDV reference
  20  CGTATTATTGTCTGGCTGCAGAGATNACTCACACTCACATCAATACTNAGCACTTGGTGT
 661' TGTGATATTATCTGGTTGCAGAGATCACTCACACTCACATCAATACTTAGCACTTGGTGT
  80  GCTTCGGACATCTGCAACAGGNAGGGTATTCTTTTCTACTCTGCGTTCCATCAATTNAGA
 721  GCTTCGGACATCTGCAACAGGGAGGGTATTCTTTTCTACTCTGCGTTCCATAAATTTAGA
 140  TGACACCCAAAATCGGAAGTCTTGCAGTGTGAGTGCAACTCCTCTAGGTTGTGATATACT
 781" TGACACCCAAAATCGGAAGTCCTGCAGTGTGAGTGCAACCCCTTTAGGTTGTGATATGCT
 200  GTGCTCCAAAGTCACAGAGACTGAAGAAGAGGATTATAAGTNAGTNNCCCCCACATCAAT
 841  GTGCTCTAAAGTCACAGAGACTGAGGAAGAGGATTACAAGTCAGTTACCCCCACATCAAT
 260  NGTGCACGGAAGGTTAGGTTTTGACGGTCAATACCATGAGAAGGACTTAGACATTACAGT
 901  GGTGCACGGAAGGCTAGGGTTTGACGGTCAATACCATGAGAAGGACTTGGACACCACAGT
 320  CTTATTTAAGGATTGGGTGGCAAATNNCCCGGGAGNGGGANGCGGATCTTTYATTAAYGA
 961  CTTATTTAAGGATTGGGTGGCAAATTACCCAGGGGTGGGAGGAGGGTCTTTTATTGACGA
 380  CCGYGYAYGGTTCCCAGTTYACGGAGGGCTAAAACCTAATTCGCCACTAGCCACTGCACA
1021' CCGTGTATGGTTCCCAGTTTACGGAGGGCTCAAACCCAATTCACCCAGTGACACTGCACA
 440  AGAAGGGAAATAYGTAAYATACAAGCGCTATAATAACACATNCCCCGATGGACAAGAATA
1081  AGAAGGGAAATATGTAATATACAAGCGCTATAATAACACATGCCCCGATGAACAAGATTA
 500  TCAAATCCGGATGGCTAAGTCTTCGTACAAGCCTGGGC-ATTCGGTGGAAAGCGTGTACA
1141  CCAAATTCGGATGGCTAAGTCTTCATATAAACCTGGGCGATTTGGTGGAAAGCGCGTACA
 559  GCAAGCCATCTTATCTATCAAAGTGTCAACATCTTTAGGTGAGGACCCGGTGTTGACTGT
1201  GCAGGCCATCTTATCTATCAAAGTGTCAACATCTTTGGGTGAGGACCCGGTGCTGACTAT
 619' TCCGCCTAATACAATTACACTCATGGGGGCCGACGG---AGTTCTCACAGTAGGGACATC
1261  TCCACCTAATACAATTACACTCATGGGGGCTGAAGGCAGAGTTCTCACAGTAGGCACATC
```

Fig 18 (Continuation)

Alignment of APMV-1/show pigeon/1831/84 sequenced RNA portion with the HN gene of the Newcatle disease virus

676	TCATTTCTTGTACCAACGAGGGTCTTCGTATTTCTCCCCTGCCTTATTATATCCTATGAC	APMV-1 isolate
1321	TCACTTCTTGTACCAACGAGGGTCTTCATATTTCTCCCCTGCTTTATTATACCCCATGAC	NDV reference
736	AGTCTATAACAAAACG	
1381	AGTAAATAACAAAACAGCTACTCTTCATAGTCCTTATACGTTTAATGCCTTCACTCGGCC	

Fig 19

APMV-3a/Turkey/Wisconsin/65

```
=====================================================================
HUSAR FASTN Program,    962 bases versus EMBL viral library
=====================================================================
The best score is with NDV (Miyadera strain) fusion (F) gene, 47 4% identity
```

Alignment the sequenced piece of APMV-3a/Turkey/Wisconsin/65 and NDV F gene

```
   1'                                        TTATACCCGACTGTGTGGTCA-A    <--- Ty/Wisc
 961" CTCGTCTGTAAGCACAACCAAGGGCTTTGCCTCGGCACTTGTCCCAAAAGTGGTAACACA    <--- NDV (EMBL)
  23' GAAGGGAGTAATAATTGAGGGAATGCTTAAGAGTAATTGTATAGAATTGGAACGAGATAT
1021" GGTCGGTTCTGTGATAGAAGAACTTGACACCTCATACTGTATAGAGACCGATCTGGATTT
  83' AATTTGCAAGACTATCAACACATACCAAATAACTAAGGAAACTAGAGCATGCTTACAAGG
1081" GTATTGTACAAGAATAGTGACATTCCCTATGTCTCCTGGTATTTATTCCTGTTTGACCGG
 143' TAATATAACAATGTGTAAGTACCAGCAGTC-CAGGACACAGTTGAGCACCCCCTTTATTA
1141" CAATACATCGGCTTGTATGTATTCAAAGACTGAAGGTGCACTT-ACTACGCCATACATGA
 202  CATATAATGGAGTTGTAATTGCAAATTGTGATTTGGTATCATGCCGATGCATAAGACCCC
1200  CTCTCAAAGGCTCAGTTATTGCAAATTGCAAGATGACAACATGTAGATGTGCAGACCCCC
 262' CTATGATTATCACACAAGTAAAAGGTTACCCTCCTGACAATTATAAATAGGAANNNATGT
1260' CGGGTATCATATCGCAAAATTATGGAGAAGCT-GTGTCTCTAATAGATAGGCACTCATGT
 322' ACCGAGTTGT-CGNGGATAATTTAATTTNAAATATTGAAACNAACCATAACTTTTCATTA
1319" AATGTCTTATCCTTAGATGGGATAACTTTGAGGCTCAGTGGGGAATTTGATGCAGCTTAT
 381' AACCCTACTATTATAGATTCACAATCCCGGCTTATAGCTACTAGTCCAT-TAGAAATAGA
1379' CAAAAGAATGTCTCAATACTAAATTCTCAAGTAATAGTGAC-AGGCAATCTCGATATCTC
 440' TGCCCTTATTCAAGATGCGCAACATCACG---CGGCTGGCCCTTCTTAAAGTAGAAGAAA
1438" AACTGAGCTTGGGAATGC-CAACAACTCGATAAGTAATGCTTTGAATAAGTTAGAGGAAA
 497' GCAATGCTCACTTATTAAGAGTTACAGGGCTGGGCTCATCAAGTTGGCACATCATACTTA
1497" GCAACAGCAAACTAGACAAAGTCA-ATGTC-AGACTGACCAA-----CACATC-TGCTCT
 557' TATTAACATTGCTTGTATGCACCATAGCATGGCTCATTGGT-TTAT-CTATTTATGTCTG
1549" CATT-ACCTATATCGTTTTAACTGTCATATCTCTTGTTTGTGGTATACTCAGCCTGGTTC
```

Fig 19

Continuation

615' CCGCATTAAAAATGATG-ACTCGACCGACAAAGAACCTACAACCCAATCATCGAACCGCG

1608" TAGCATGCTACCTGATGCACAAGCAAAAGGCGCAACAAAAGACCTTGTTATGGCTTGGGA

674' GCATTGGGGTTGGATCTATACAATATATGACATAATGNGCCGCCTGTATATCANNNCCAA

1668" ATAAT-ACCCTAGATC-AGATGAGAGCTACCACAAAAGCATGAATGTAGATGAGAGGC-A

734' GTATCGNNNCCTCCCACCATCCTCNNCGCCGCCACTAGCAGCACAGGAAGTAATCAGTTA

1725' TAGGCATCCCCAACCGCAAT-TTGTGTGTAAATTCTGGCAGC-CTGTCA---ATCAGAAG

794' CAGTGGCATAGCAGTCCCATGTTGAGACACACAGTACCCCTAGTTTCTAGTAAANNNCCG

1780" AAT TAAG AAAAAA CTACCGGGTATAGATGACCAAAGGGCAATAT

The END of the F gene of NDV

The START of the HN gene of NDV

Fig 20 Alignment of APMV-3a/turkey/Wisconsin/65 versus APMV-3b/parakeet/Netherlands/1975

```
X         10         20         30         40         50
CATAGTGTgCAAAGGyAkCsGACsaAgtCTcTCtgnTCywGAcabaGGCGwCGyaCACyA     Tw/Wisc

                                        GAnACAGAAGttGGTGAGnnAC     Pk/Neth
                                        X         10         20

          70         80         90         100        110
CCCmwAATAakt-bAGpCCGACATAGCAyCAATcaGGtAcATGCAGGcGGCCCTAGtagG

CCTTCCTCAAaTnAAGACTGAnATtGCTTCGATTCGGAACAtGCAAGCAGCTTTGGAAGG
       30         40         50         60         70         80

120        130        140        150        160        170
TcAAATTAgCATGATAAGGATaCTCnGACCCCnGCaACaACacAGAGTCATCCCTAAATA

ACAGCTCAGnATGATTCGnAnATtGGAtCCAGGA-AACTGTTCGGAATCATCACTCAAtG
       90         100        110        120        130

180        190        200        210        220        230
CCCTCCGCAAC^CtgGAAATcGGGCTCCAGTAGtGATTTGCGGACCGGGCgaCCCTCACC

CGCTGAGAGGCtTCAGTGCnAAGGCACCTGTnATTGTGaGTGGTCCAGGAAATCCCAATC
        150        160        170        180        190

240        250        260        270        280        290
GCAGTCTGATCAAAAGCGAGAACCCGACTATCTGCCTGGATGAACTAGCtCGGCCAACTC

AATGCATCAGCCAAGGCTCATCCACCACAATTtGCCTGGATGAGTtAGCCCGGCCTGTGC
        210        220        230        240        250

300        310        320        330        340        350
AAGCCAACAGTCCTCCAAAATCTCAAGATAACCAAaGGGATcTtTCCGCTCAACGACACG

CAAATCCtAtACAGGTGAAACTTCAAGATGATCAGAAGGACCtATCTGCACAGCgACATG
        270        280        290        300        310

360        370        380        390        400        410
CAATCACAGCTCTGCTAGAAacCCgCGTTGCACCcGGACCTAAGAGAGATCGCCTGATGG

CT-TCATtGCATnACTCgAaACcAGaAnncC-CCCGGACCAAaACGtGACAAATnACtGT
         330        340        350        360        370

420        430        440        450        460        470
AAATgGTAGTAGCAGCGAAATCAGCAAGTGATCTCATCAAAGTCAAGAGAATGGCAATTC

CATtgGThGCAATtGCAAAgACTGCAAGhGATThAATCAAGAThaAGAGAAtGGCA----
380        390        400        410        420        430
```

Fig 20 Continuation

```
480        490        500        510        520        530
TTGGTCAAtAAACCGAcTcAGCACCACATnGTCTGTGACTCTACACTTGTGCGGCAAACC

---GTCCTCGGCCAGhGAhATCACCChATGGCAACcAGGCTACAAATGTAAGCAGTTTCC
       440        450        460        470        480        490

540        550        560                   570        580
AACATTGACCTCCAAACACTT--------TTCTGCAGTACGCAAGGCTTAACACAATcAG

GATATGTACCGAATCAATAACACnACCCCTTCCGCAAAAGCAAGCGCGAGATGACACACT
       500        510        520        530        540        550

        600        610        620        630        640
CAGCATGCATATCGAGCGGCCCACCCTCACAACCCATCTAGCTCTCTTATTTTATCTATT

CAGCTTnACCAATTCTAGCTTCACTAAGCAGGTG-ATCTGACCATCTGGCTTTACTTCC-
       560        570        580        590        600

        660        670        680        690        700
GCTTTATAAAAAACnAAAATGATTATAACTAAAgAATCTCAACAATTTGCAATGATAACA

GCTTTATAAAAAACTCCTAGATCTAACATGGAGGCAGACCGAGCTAAtGCgGAGGGAGTA
         620        630        640        650        660

        720        730        740        750        760
ACACCATACGATCACTAGGGGCGGACGAAATAACCCAAGGACCAATCTCCGAGTCCAGGC

CACAnngGGAATCAAAggA
         680

        780        790        800        810        820
CAGACACAGGCAaCCCATcAGCAcAgAGCCAAGCAACCAAAAtgGCAGCACACCCcA
```

Fig 21 Sequence of the portion of the APMV-3a/Turkey/Wisconsin/65 virus obtained by annealing with a primer to the F gene of the NDV

CATAGTGTgCAAAGGyAkCsGACsaAgtCTcTCtgnTCywGAcabaGGCGwCGyaCACyA

CCCmwAATAaktbAGpCCGACATAGCAyCAATcaGGtAcATGCAGGcGGCCCTAGtagGT

cAAATTAgCATGATAAGGATaCTCnGACCCCnGCaACaACacAGAGTCATCCCTAAATAC

CCTCCGCAACTCtgGAAATcGGGCTCCAGTAGtGATTTGCGGACCGGGCgaCCCTCACCG

CAGTCTGATCAAAAGCGAGAACCCGACTATCTGCCTGGATGAACTAGCtCGGCCAACTCA

AGCCAACAGTCCTCCAAAATCTCAAGATAACCAAaGGGATcTtTCCGCTCAACGACACGC

AATCACAGCTCTGCTAGAAacCCgCGTTGCACCcCGACCTAAGAGAGATCGCCTGATGGA

AATgGTAGTAGCAGCGAAATCAGCAAGTGATCTCATCAAAGTCAAGAGAATGGCAATTCT

TGGTCAAtAAACCGAcTcAGCACCACATnGTCTGTGACTCTACACTTGTGCGGCAAACCA

ACATTGACCTCCAAACACTTTTCTGCAGTACGCAAGGCTTAACACAATcAGCAGCATGCAT

ATCGAGCGGCCCACCCTCACAACCCATCTAGCTCTCTTATTTTATCTATTGCTTTATAAA

AAACnAAAATGATTATAACTAAAgAATCTCAACAATTTGCAATGATAACAACACCATACG

ATCACTAGGCGCGGACGAAATAACCCAAGGACCAATCTCCGAGTCCAGGCCAGACACAGG

CAACCCATC AGCACAGAGC CAAGCAACCA AAATGGCAGC ACACCCC

Fig 22

Sequence of the end-portion of the genome of
APMV-3a/Turkey/Wisconsin/67, 695 nucleotidess

aAGACaTGCA AAACAGAGaA GGCGTACACC ATCTGAAACC ACTCaAAAGG TGCGCAGAAT

GGgTTTCGGA TTGGACTCTA CwATCTTTbG GGCCCTACAA TAGTGGTCGA ATTAACTGGC

TCGTCGCGcA CTCACTCGCT CCAGGGATTC TTCTCCAGTG AGAGACTsGC TTGCTCCTAT

TTCACAATAC aACCCACATG TCGGwCAACt GATTTGGGCA CATGATGTTT CAATAACAGG

CTGTCATAtG ATAATATCTG AaCTTGAGAA AAAGadAGCT TTGGCCATGs TGCACCTCAC

tGTAAGGATG CAGTTGCTAT CAATACTACA ATAAAGGAGT TGGTTCCTTT CCGCThGTTC

AGGAAATAAA TcACTCACTG CCGCCAGCTT ACCACTAGTA ACAAATnACA ACCATCACCT

ATAACCTAAC AAACCAAAwG CAtGCACCTA ACCTTCTGGG TTGAAtGAGA AGCTTGGATT

ATATTCATGA TnAGCTAACA CGAATTtATT GCTTAAATTG CTTATACCGG TAATAACTCA

AATATTCCAC TAACCAAATT TAATTAAAAA tATtAATAAT sATnAGCAAC ATCCGATCGG

AAtCTTCAGG GGCGGAAGGn CnACcGCCAC AAnnnnCCpC CACACCAGGC GGGnCCGnnn

nCCCACAGGA CnGGCCACAC CAAACAAAGn nnnnn

Fig 23

Sequence of the end-portion of the genome of APMV-3a/Turkey/Wisconsin/67, fluorogram

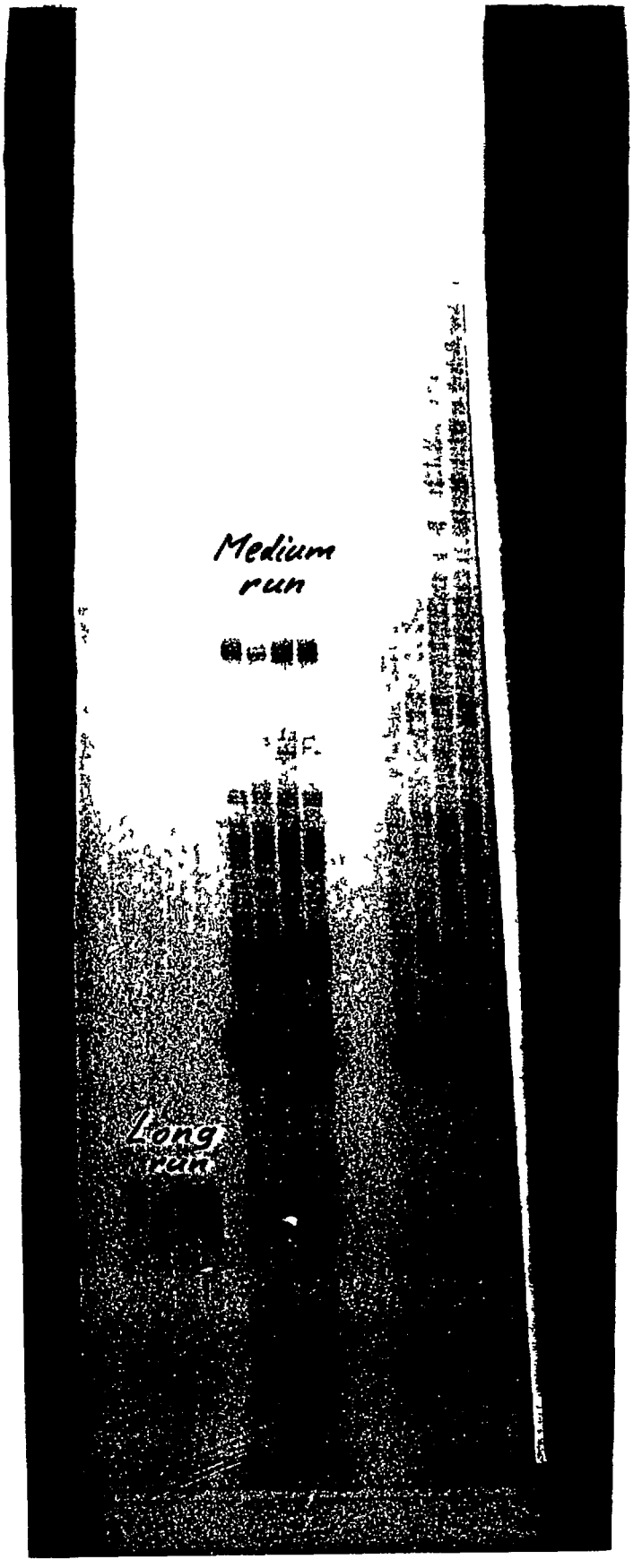


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Fig 24

APMV-3b/Parakeet/Netherlands/449/75

```
==================================================================
DP5 Program      Open reading frame (ORF) of P gene        687 bases
==================================================================

                          Amino acid sequence

  1 G ANA CAG AAG TTG GTG AGN ACC CTT CCT CAA ATN AAG ACT GAN ATT GCT
      ??? Gln Lys Leu Val ??? Thr Leu Pro Gln ??? Lys Thr ??? Ile Ala

 49   TCG ATT CGG AAC ATG CAA GCA GCT TTG GAA GGA CAG CTC AGN ATG ATT
      Ser Ile Arg Asn MET Gln Ala Ala Leu Glu Gly Gln Leu ??? MET Ile

 97   CGN ANA TTG GAT CCA GGA AAC TGT TCG GAA TCA TCA CTC AAT GCG CTG
      Arg ??? Leu Asp Pro Gly Asn Cys Ser Glu Ser Ser Leu Asn Ala Leu

145   AGA GGG TTC AGT GGN AAG GCA CCT GTN ATT GTG AGT GGT CCA GGA AAT
      Arg Gly Phe Ser Gly Lys Ala Pro Val Ile Val Ser Gly Pro Gly Asn

193   CCC AAT CAA TGC ATC AGC CAA GGC TCA TCC ACC ACA ATT TGC CTG GAT
      Pro Asn Gln Cys Ile Ser Gln Gly Ser Ser Thr Thr Ile Cys Leu Asp

241   GAG TTA GCC CGG CCT GTG CCA AAT CCT ATA CAG GTG AAA CTT CAA GAT
      Glu Leu Ala Arg Pro Val Pro Asn Pro Ile Gln Val Lys Leu Gln Asp

289   GAT CAG AAG GAC CTA TCT GCA CAG CGA CAT GCT GTC ATT GCA TNA CTC
      Asp Gln Lys Asp Leu Ser Ala Gln Arg His Ala Val Ile Ala ??? Leu

337   GAA ACC AGA ANN NCC CCG GAC CAA AAC GTG ACA AAT NAC TGT CAT TGG
      Glu Thr Arg ??? ??? Pro Asp Gln Asn Val Thr Asn ??? Cys His Trp

385   TNG CAA TTG CAA AGA CTG CAA GNG ATT NAA TCA AGA TNA AGA GAA TGG
      ??? Gln Leu Gln Arg Leu Gln ??? Ile ??? Ser Arg ??? Arg Glu Trp

433   CAG TCC TCG GCC AGN GAN ATC ACC CNA TGG CAA CCA GGC NAC AAA TGA
      Gln Ser Ser Ala ??? ??? Ile Thr ??? Trp Gln Pro Gly ??? Lys TER

481   AAGCAGTTTCCGANATGNACCGAATCAANAACACNACCCCTTCCGCAAAAGNNAGCGCGCGNC

544   CACACACTNAGCTTNANCAATTCTAGCTTCACNAAGCAGGTGATCTGACCATCTGGCTTNACT
            TATA box
607   TCCGCT TAAG AAAAAA CT CCTAGATCNA ANATGGAGGCGGNCCGAGCNAATNGAAGAG
                 Poly-A    Concensus?
    -<=----------------    -------=>-
  Putative END of P gene   Putative START of M gene

666   AGNACACAAANGGGGGTCGGGG     686
```

Fig 25

PMV-3/Swine/Nazareth/80

```
==================================================================
HUSAR  FASTN        668 bases versus EMBL viral library
==================================================================
The best score is with Mumps virus, Miyahara strain, P gene, 48.7% identity
------------------------------------------------------------------

ALIGNMENT  the sequenced piece of PMV-3/Swine/Nazareth/80 and mumps virus P gene

   1'                                                  ACAGAAGTTGGTGAGN
                                                                  .  ..
 841" ATGGATGCTCGCCTGCAACATCTTGAACAAAAGGTGGACAAGGTGCTTGCACAGGGCAGC

  17' ACCCTTCCTCAAATNAAGACTGANATTGCTTCGATTCGGAACATGCAAGCAGCTTNGGAA
      :   .                  . .            :.            :     .
 901" ATGGTGACCCAAATAAAGAATGAATTATCAACAGTAAAGACAACACTAGCTACAATTGAA

  77' GGACAGCTCAGNATGATTCGNANATTGGATCCAGGAAACTGTTCGGAATCATCACTCAAT
      ..     .  .                . ..'  . :. '    :          : :   .
 961" GGAATGATGGCGACAGTAAAGATCATGGATCCTGGAAACCCGACAGGGGTCCCAGTTGAT

 137' GCGCTGAG---AGGGTTCAGYGGYAAGGCACCTGTNATTGTGAGYGGTCCAGGAAATCCC
      .            : .   : ' . :     .      ' :   .   : :'.: :
1021" GAGCTTAGAAGAAGTTTTAGTGATCATGTAAC---AATTGTTAGTGGACCAGGAGAT--G

 194' AATCAATGCATCAGCCAAGGCTCATCCACCACAATTTGCCTGGATGAGTNNGCCCNGCCT
                          .   .     .  .  .     .  .     .  :     .
1076" TGTC-AT---TCAGCTCCGGTGAAGAACCCACACTGTATTTGGATGAACTAGCGAGGCCT

 254' GTGCCAAATCCNANACAGGTGAAAC-TTCAAGA---TGATCAG--AAGGACCNATCTGCA
      .                   .         .          :       .
1132" GTCCCAAAGCCCCGTCCTGCAAAGCAGCCAAAACCCCAACCAGTAAAGGATTTAGCAGGA

 308' CAGCGACATGCTG-TCATTGCATNACTCGAAACNAGNANNNCCCCGGACNAAAACGTGAC
      .          .  .  .        .              .       :     . .'  .
1192" C-GGAAAGTGATGATAACTAAAATGATC-ACTGACTGTGTGGCCAATCCTCAAATGAAGC

 367' AAATNACTG-TCATTGGTT-GCAATTGCAAAGACTGCAAGTGATTNAATCAAGATNNAGA
                          .  .     '.     .          .          :.  '.:
1250" AGGTGTTTGAGCAACGATTGGCAAAGGCCAGCACCGAGGATGCTCTGAATGATATCAAGC

 425' GA-ATNGCA-GTCCTCCGCCAGTGATATCACCCTATGGCAACNAGGCNACAAATGNAAGC
      :                   .. . .        .   . :   .   .          .
1310" GAGACATCATAAGAAGCGCCA-TATGAACTCACCAGGAACACCAGACT-CACGGGAAAAT

 483' AGTTTCCGATATGTACCGAATCAATAACACNACCCCTTCCGCAANAGNNAGCGCGCGNCC
                          : . .     .            .. :  ..      543'
1368" CCACAAACTGAAAGCCACAATGATTCCCTGTTTAATAAAAAAAAAAAAAA 1417"
                                   -<=---------------=>-
                    END of the P gene of Mumps (Miyahara)
```

Fig 26

```
ALIGNMENT        OF      APMV-3b/Parakeet/Netherlands/75  687 BASES
                VERSUS    PMV-3b/Swine/Nazareth/80        668 BASES
==================================================================
NUCALN Program
------------------------------------------------------------------

X        10        20        30        40        50
ANACAGAAGTTGGTGAGNACCCTTCCTCAAATNAAGACTGANATTGCTTCGATTCGGAAC  Pk/Neth
   CAGAAGTTGGTGAGNACCCTTCCTCAAATNAAGACTGANATTGCTTCGATTCGGAAC  Sw/Naz

61  ATGCAAGCAGCTTTGGAAGGACAGCTCAGNATGATTCGNANATTGGATCCAGGAAACTGT  Pk
    ATGCAAGCAGCTTNGGAAGGACAGCTCAGNATGATTCGNANATTGGATCCAGGAAACTGT  Sw

120 TCGGAATCATCACTCAATGCGCTGAGAGGGTTCAGTGGNAAGGCACCTGTNATTGTGAGT  Pk
    TCGGAATCATCACTCAATGCGCTGAGAGGGTTCAGYGGYAAGGCACCTGTNATTGTGAGY  Sw

181 GGTCCAGGAAATCCCAATCAATGCATCAGCCAAGGCTCATCCACCACAATTTGCCTGGAT  Pk
    GGTCCAGGAAATCCCAATCAATGCATCAGCCAAGGCTCATCCACCACAATTTGCCTGGAT  Sw

241 GAGTTAGCCCGGCCTGTGCCAAATCCTATACAGGTGAAACTTCAAGATGATCAGAAGGAC  Pk
    GAGTNNGCCCNGCCTGTGCCAAATCCNANACAGGTGAAACTTCAAGATGATCAGAAGGAC  Sw

301 CTATCTGCACAGCGACATGCTGTCATTGCATNACTCGAAACCAGAANNNCCCCGGACCAA  Pk
    CNATCTGCACAGCGACATGCTGTCATTGCATNACTCGAAACNAGNANNNCCCCGGACNAA  Sw

361 AACGTGACAAATNACTGTCATTGGTNGCAATTGCAAAGACTGCAAGNGATTNAATCAAGA  Pk
    AACGTGACAAATNACTGTCATTGGTTGCAATTGCAAAGACTGCAAGTGATTNAATCAAGA  Sw

421 TNAAGAGAATGGCAGTCCTCGGCCAGNGANATCACCCNATGGCAACCAGGCNACAAANGN  Pk
    TNMAGAGAATNGCAGTCCTCCGCCAGTGATATCACCCTATGGCAACNAGGCNACAAATGN  Sw

481 AAGCAGTTTCCGANATGNACCGAATCAANAACACNACCCCTTCCGCAAAAGNNAGCGCGC  Pk
    AAGCAGTTTCCGATATGTACCGAATCAATAACACNACCCCTTCCGCAANAGNNAGCGCGC  Sw

541 GNCCACACACTNAGCTTNANCAATTCTAGCTTCACNAAGCAGGTGATCTGACCATCTGGC  Pk
    GATGACACACTNAGCTTNAGCAATTCtAGCTTCACTAAGCAGGTGATCTGACNATCTGGC  Sw

601 TTNACTTCCGCT TAAG AAAAAA CT CCTAGATCNAANATGGAGGCGGNC      Pk/Neth
    TTNACTTCCGCT TAAG AAAANA CT CCTAGATCNAANATGGAGGTAGAC      Sw/Naz
            CCTAT TAAATAAAAAA AA GCACGAACACAA                 Mumps/SBL
```

Fig 26

Continuation

```
661 CGAGCNAATNGAAGAGAGNACACAAANGGGGGTCGGGG   687   Pk/Neth
    NPGCT-AATGGNNGAGTACACAN                  668   Sw/Naz
```

NUMBER OF MATCHED BASES=624

Percentage of homology MINIMAL = 93 4%

Fig 27

Sequence of the END portion of the genome of the APMV-6/duck/Hong Kong/D199/77, 545 nucleotides

AAGACAATng CCGCGCGTGC AACCAAGGAA CATATTGCAT CTACATATAG TTAGACTTAT
TGGAGACTCA ATGTCCAATG TAAGGAGACT AGTACCTCTG CTAGATAAGG GCTTTATAGT
AATATCAGAC ATGTATAGTG TGAAAGATTT CTTGAGAAAA ACTGAGTCCC CCTAAGTACT
TCTTAAACAA GCnaGGCAAG AGCGAGATTG CACACCTATT TGAGAtAGAG TCnAAGAThA
TTCTgaGCAG GGcapAGATn AAGAAnATTT TGmAgACAAy ngGGATTGTG GCTAAAgnGc
ACTcAGAGTg ATCTCTCCAA CCTTGCACcA TTTgAATTCT GGACTGTggA CgCGCAhcCt
aAGCGcACca aCTnGCCGTG ACGATTnATG yAATCCTTGA tATGAACnaC naATCATTTG
GAATTnATTn ACTTCCCGAA ATcACCCAtA GnCCGGAATC GATACCGGAG ATtATTTTTn
AAbAAAAnaC CTGGAAAGTC nAcnAGGATc ATAGTCAAAn AGCTtATGAT TyCCTtgTTT
Gnnnn

Fig 28

Sequence of the END portion of the genome of the APMV-6/duck/Hong Kong/D199/77, fluorogram

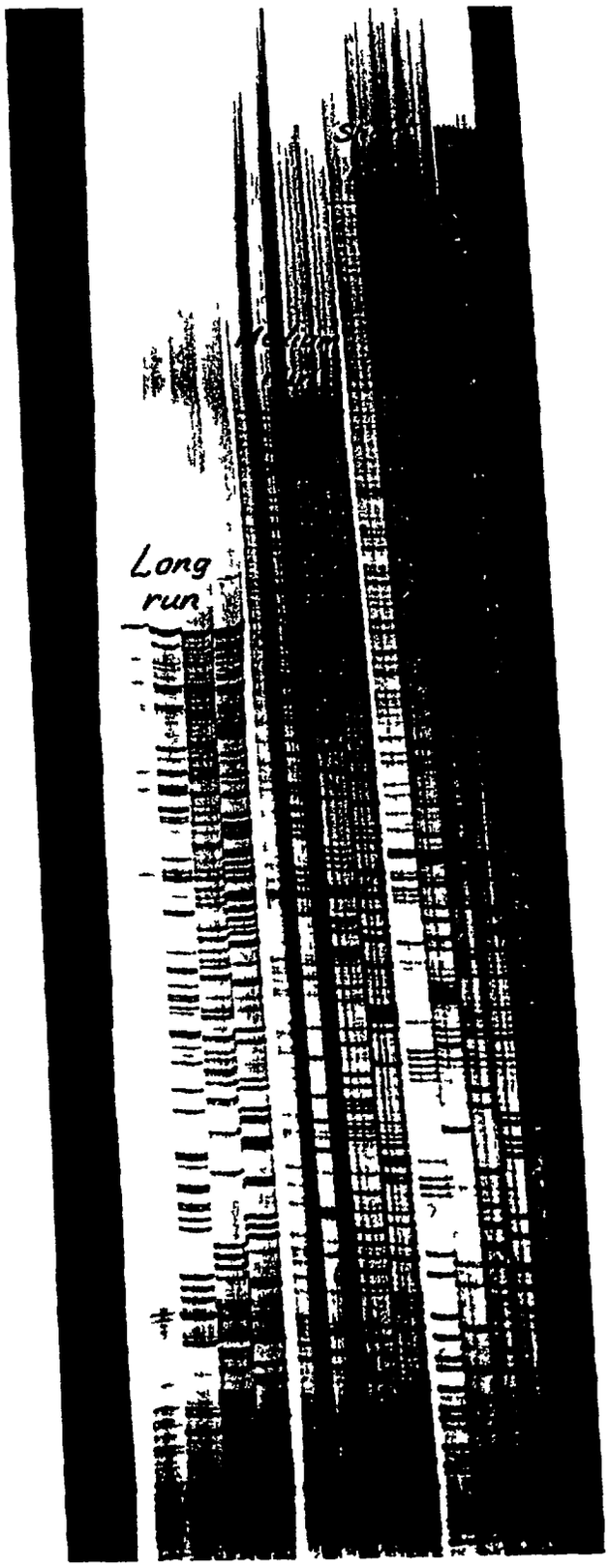

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Fig 29

PMV-7/Dove/Tennessee/77

```
==================================================================
HUSAR FASTN Program     971 bases versus EMBL viral library
==================================================================
```

The best score is with Human parainfluenza virus type 2 matrix (M) gene, 50 9% identity

---

Alignment the sequenced piece of PMV-7/Dove/Tennessee/77 and PIV-2 M gene

```
  1'   GTGCCAGCGGGTATGCTGACATTGGGCCAAATAAGGACCAATATAGACCCGGACGAAA   <--- Dove/Tn
                                                  .. .     . . . .
361" CCATAACAGCATGCATGATGAAGCTAGG----ACCTGGTCCAA-ATA-TTCAGAATGCAA   <--- PIV-2 (EMBL)

 59' TTGGAGAGGCATGCT-TGGGACTCAA----AGGGAATGCTAAAAAAG-GAGC-GGCAAGT
                                                  .    .        .
415" ATCTAGTGCTAAGATCTCTGAATGAATTCTACGTAAAAGTCAAGAAGACATCAAGCCAGA

112' GAGGAGAAGATAGTATTTAGCATTCTGGAAAAGCCTCCCGCCCGGGGGACTGCA--CCTG
     . .         ..    . .       . .                .     :  .
475" GAGAGGAAG-CAGTGTTT-GAATTAGTTAACATTCCAACTTTATTGAGAGAACATGCTCT

170' TAGTACAAGAAAGGGGCTTAATGGCTAAAGCAGAAGGATCAATCAAATGCCCAGGTAAGA
     .                                 . .
533" TTGCAAACGCAAAATG-TTAGTATGCTCTGCAGAAAAATTCCTCAAGAACCCGTCAAAGC

230' TGATGAGTGAAATTNACNACTCATTTAGAGTAATGTTTGTGAGTATCACAATGCTGGATA
                                     .                .     .
592" TACAAGCTGGATTTGAGTATGTATACATACCAACTTTTGTCTCCATTAC-ATACTCACCA

290' ATCAGAGCCTA-TACAGAGNACCAACAGCCATCAGCTCGTTCAAAAATAAAGCTCTATAT
                                       :       .  ..            .
651" CGAAATCTGAATTACCAAGTGGCCAGACCTATCCTTAAGTTCAGATCACGCTTTGTGTAT

349' TCTATTCAGTTAGAGGTATTGCTGGAAGTTGATGTG-AAGCCTGAGAGCCCCCAG-TGTA
          .         .                .         .     :
711" AGCATTCATTTGGAATTAATCCT-GAGATTGCTATGCAAATCTGACTCCCCCTTGATGAA

407' A-ATTTCTA-GCAGACCAGAAAGG--GAAGA-AAGTTGCTTCTGTATGGTTCCATCTCTG
                                                        .
770" ATCCTACAATGCAGACAGAACAGGTCGGGGATGCCTCGCATCAGTCTGGATCCATGTATG

462' CAA--TTCTAAAAAGACGAATGCCAGYNGGAAACCGAGATCATTAGAGGATATGAGAAAG
                                        .                       .
830" TAACATTCTGAAAA-ACAAAAG-CATCAAGCAACAAGGCAGAGAATCATATTTCATAGCT

520' NAGGTCCGAGATATGGGAATCAAAGTGTCTCTGGCCGACCTTTGGGGCCCTACGATCATC
                 .                   .  .        .           .  .  .
888" AAGTGCATGAGCATGCAGCTGCAGGTGTCCATTGCAGATCTTTGGGGACCAACAATCATA

580' GTCAGGGCCACAGGGAAGATGAGTAAATATATGCTAGGATTTTTCTCTACCTCAGGGACT
          .          .                .                        . ..
948" ATCAAATCATTGGGTCACATCCCCAAGACTGCACTTCCTTTTTTCAGCAAAGATGGGATT
```

Fig 29

Continuation

```
 640' TCATGTCATCCAGTAACAAAGAGTTCACCAGATTTGGCAAAAATATTATGGTCATGCTCA
1008" GCCTGTCATCCATTACAAGATGTTTCCCCTAATCTAGCAAAATCACTGTGGTCAGTTGGA

 700' AGCACNATNATCAAAGCAAATGCCATTGTTCAAGGGTCAG-TCAAAGTCGATGTCCTGAC
1068" TGTGAGATAGAATCTGCCAAGTTGATACTTCAAGAATCTGATCTTAAT-GAGCTAATGGG

 759' CCTCGAAGA---TATCCAAGTTTCCAGTG----CTGCAAAAATCAAC--AAATNAGGAAT
1127" CCACCAGGACCTTATCACTGATAAGATTGCCATTAGATCAGGTCAACGGACATTTGAGAG

 810' AGGGAAGTTTAATCCATTTAAGAAATA-AAGTCATATGCAGATTAAAATTTGATCAA--G
1187" GTCCAAATTCAGCCCATTCAAAAAATATGCATCAATTCCAAACTTGGAAGCCATCAACTG

 867' ATTGGTCTTAGCA-AACTTAACTGAACTGTAATTATAAAANACCTC-AGTAAAATGCTAA
1247" AATGCTC-CAGCATCTGAGAATAGAACCACAATCAAGTCATACTACTAGTCACTATACAA

 925' TGAATCAGTGGATGATATTGAATNAGCAGATTGAAAATTAAAGAAAC
1306" T-AATCA----ACAAT-TTTAGTCAACTGATTACCAAGATGTTATCATAGGTCCGAACTG
```

Fig 30

APMV-7/Dove/Tennessee M Gene mRNA-sense ORF

Sequence dov-d-3v sf3, variant Dov-d-3v trv
Length 971

Frame 1

Range	Nucl. Length	Polypeptide Length	Type of orf
1 to 834	834	278	5'end-Ter

5'-
GTG CCA GCG GGT ATG CTG ACA TTG GGC CAA ATA AGG ACC AAT ATA GAC CCG GAC GAA ATT GGA GAG
Val Pro Ala Gly MET Leu Thr Leu Gly Gln Ile Arg Thr Asn Ile Asp Pro Asp Glu Ile Gly Glu

GCA TGC TTG GGA CTC AAA GGG AAT GCT AAA AAA GGA GCG GCA AGT GAG GAG AAG ATA GTA TTT AGC
Ala Cys Leu Gly Leu Lys Gly Asn Ala Lys Lys Gly Ala Ala Ser Glu Glu Lys Ile Val Phe Ser

ATT CTG GAA AAG CCT CCC GCC CGG GGG ACT GCA CCT GTA GTA CAA GAA AGG GGC TTA ATG GCT AAA
Ile Leu Glu Lys Pro Pro Ala Arg Gly Thr Ala Pro Val Val Gln Glu Arg Gly Leu MET Ala Lys

GCA GAA GGA TCA ATC AAA TGC CCA GGT AAG ATG ATG AGT GAA ATT NAC NAC TCA TTT AGA GTA ATG
Ala Glu Gly Ser Ile Lys Cys Pro Gly Lys MET MET Ser Glu Ile ??? ??? Ser Phe Arg Val MET

TTT GTG AGT ATC ACA ATG CTG GAT AAT CAG AGC CTA TAC AGA GBA CCA ACA GCC ATC AGC TCG TTC
Phe Val Ser Ile Thr MET Leu Asp Asn Gln Ser Leu Tyr Arg ??? Pro Thr Ala Ile Ser Ser Phe

AAA AAT AAA GCT CTA TAT TCT ATT CAG TTA GAG GTA TTG CTG GAA GTT GAT GTG AAG CCT GAG AGC
Lys Asn Lys Ala Leu Tyr Ser Ile Gln Leu Glu Val Leu Leu Glu Val Asp Val Lys Pro Glu Ser

CCC CAG TGT AAA TTT CTA GCA GAC CAG AAA GGG AAG AAA GTT GCT TCT GTA TGG TTC CAT CTC TGC
Pro Gln Cys Lys Phe Leu Ala Asp Gln Lys Gly Lys Lys Val Ala Ser Val Trp Phe His Leu Cys

AAT TCT AAA AAG ACG AAT GCC AGY NGG AAA CCG AGA TCA TTA GAG GAT ATG AGA AAG NAG GTC CGA
Asn Ser Lys Lys Thr Asn Ala Ser ??? Lys Pro Arg Ser Leu Glu Asp MET Arg Lys ??? Val Arg

GAT ATG GGA ATC AAA GTC TCT CTG GCC GAC CTT TGG GGC CCT ACG ATC ATC GTC AGG GCC ACA GGC
Asp MET Gly Ile Lys Val Ser Leu Ala Asp Leu Trp Gly Pro Thr Ile Ile Val Arg Ala Thr Gly

AAG ATG AGT AAA TAT ATG CTA GGA TTT TTC TCT ACC TCA GGG ACT TCA TGT CAT CCA GTA ACA AAG
Lys MET Ser Lys Tyr MET Leu Gly Phe Phe Ser Thr Ser Gly Thr Ser Cys His Pro Val Thr Lys

AGT TCA CCA GAT TTC CTA AAA ATA TTA TGG TCA TGC TCA AGC ACN ATN ATC AAA GCA AAT GCC ATT
Ser Ser Pro Asp Leu Ala Lys Ile Leu Trp Ser Cys Ser Ser Thr ??? Ile Lys Ala Asn Ala Ile

GTT CAA GGG TCA GTC AAA GTC GAT GTC CTG ACC CTC GAA GAT ATC CAA GTT TCC AGT GCT GCA AAA
Val Gln Gly Ser Val Lys Val Asp Val Leu Thr Leu Glu Asp Ile Gln Val Ser Ser Ala Ala Lys

ATC AAC AAA TNA GGA ATA GGG AAG TTT AAT CCA TTT AAG AAA TAA AGTCATATGCAGATTAAAATTTGATCA
Ile Asn Lys ??? Gly Ile Gly Lys Phe Asn Pro Phe Lys Lys END

AGATTGGTCTTAGCAAACTTAACT GAACTGTAAT TATA AAANA CCTCAGTAAAATGCTAATGAATCAGTGGATGATATTGAAT
TTAT AAAAN-A-

NAGCAGATTGAAAATTAAAGAAAC - 3'

147

Fig 31 Sequence of the portion of the APMV-7/dove/Tennessee/75 corresponding to the 5' portion of the F gene

ACCTcAGTAAAATGCTAATGAATcAGTGGATGATATTGAATnAGCAGATTGAAAATTAAA

GAAAACCTTATGAGGGCGAATGAGCTTAGATGATTTAATAAAGGAGACTAATCCAACATT

TCCCTCAAATTAACAAAATCAGAAAGTAAAAAGAnnnGGAGCAATGAGAGTACGACCTTT

AATAATAATCCTAGAGCTTTTGAGTTGCTGTGGATAATATTCTACCCGTAATTGGCTTAG

ACAATTCAAAGATTGCACAAGCAGGTATTATCAGTGCACAAGAATATGCAGTTAATGTGT

ATTCACAGAGTAATGAGGCTTACATTGCACTGCGCACTGTGCCATATATACCTCCACACA

TCTTCTCTTGTTTCCAGGATTATCAACACATACATACAACGATcAAAACAtATTCTCACC

AATtnAGGATCAAATnACATCTATAACATCGGCGTCAACnnnCCCTCATCAAGATTnGCA

GGATnAGtAGTCGGtGCAATCGCTCTCGGAGnAGCGAnATCTGCACAAAbnACtGCAGCC

GTGGACTCACAAdGGCAnAGCAGAACGCTCAAGAAAbAAtACGATkACGyGATTCTATCC

AAAAtACtATcAATGCTGtGAATGACATAACAGtAGGGTTAAGTTcAATAGGAGtAGCAC

tAAGCAnnGTCcAAAACTACTTGAATGATGTGATAAACCCTGCTCTGCAGaaCCTGAGCT

GCcAGGTTTCTGCATTAAACTtAGGGATCCAATnAAATCTTTATTTAACCGAAATTACAA

CtATCTTTGGACCGCAAATtACAAATCCATCATTGACCCnATTGTCAATtnaGGCATTAT

ACACCCkAGsAGGAGAnnACCTGATGCAATTTCtAACgAGGTATGGCTATGGAGAGACgA

GtGTgAGCAGtATTCTCGAGTCAGGACtAATATnAGCACAAAThGTATCTTThGATAAAC

AGAnnsGCATTGCAAtATmGyAcGTCACATTACCATnAATTGCGACTCTTTCCGGTTCtA

GAGTTACcAAATTGATGTCAGTtAGTGTCCAAACTGGAGTtGGAGAGGGaTCTGCTATTG

TCCcATcATA CGTtATTCAG CAGGGaacAG TAATAGAAGA AttA

Fig 32

Sequence of a genome portion of APMV-7/Dove/Tennessee/75, 1215 nucleotides

AGGAACCTGG AGACnnGGTA GAGTCCTTAC GATTCTATTA GATGTAGTGA TCATTATTCT
CTCTGGGTGA TTGCTCTGAT TTCATTGGGT CTGAAGCCAG GTGAGAGGAT CATCAATGAA
GTCAATnGAT CTATCCATAA TCAACTTGTT CCCTTATCGG GGATTACTTC CGATATTCAG
GCAAAAGTCA GCAGCATATA TCGGAGnAAC TTGCTAAGTA TCCCACTACA ACTTGATCAA
ATCAACnAGG CAATATCATC ATCTGCTAGG CAAATTGCTG ATACAATCAA CTCGTTTCTC
GCTCTGAATG GCAGTGGAAC TTTTATTTAT ACAAAATTTA TACAAATTcA CCTGAGTTcG
CAAATGGTTT CAATAGAGCA ATGCTTCCCA ACCCTAAATC AAAGCTTAAA TATGCTAACA
CCTGGTAATC TAATTGAATT TACTAATTTn ATTCCAACTC CAACAACAAA ATnaGGATGT
ATCAGAAtAC CATCATTTTC AATGTCATCA AGTCACTGGT GTTATACCCA TAATATCATT
GCTAGTGGAT GTCAGGATCA TTCAACcAGT AGTGAATACA TATCGATngG GGTTGTTGAA
GTGACTGATC AGGCTnnCCC GAACTTTCGG ACAACTCTTT CTATTACATt AGCTGATAAT
CTAAACAGAa AGTCATGTAG CATTGCAGCA ACTGGGTTCG GGTGtGATAT ATTAtGTAGT
GTTGTCACTG AGACAGAAAa TGATGATTAT CAATnaCnAG AACCGACTCA GATGATCTAt
GGAAGATTAT TTTTtaatGG CACATATTCA GAGaTGTCAT TGAATGTGAA CcAAATGTTC
GCAGATTGGG TTGCAAATyA TCnAGCAGTT GGATcAGGAG yAgAGTyAGC AGATTTTGTC
ATTTTCCCAC TCTATGGAGG TGTTAAAATc ACTnnaACCC TAGGAGCATC TTTAAGCCAG
TATTACTATA TTCCCAAGGT GCCCACAGTC AATTGCTCTG AGACAGATGC ACAACAAAyA
GAGAAGGCAA AAGCATCCTA TTCACcACCT AAAGTGCTCC AAATATCTGG GCTCAGGCAG
TCGTTAGGTG CAATAAATCT GTcAATCTTG CAAATTCATG TGAAATtCTG ACATTnnAnA
CnaGCACTAT GATGATGGGT GCTGAGGGAA GACtCGTGAT GATAGGAAGa ATGCTATACT
TTnAATGCGA ACGAT

Fig 33

Alignment of the sequenced portion of the APMV-7/dove/Tennessee/75 with NDV HN gene, 49 6% identity in 1215 overlapping

```
  1          AGGAACCTGGAGACNNGGTAGAGTCCTTACGATTCTATTAGATGTAGTGATC    APMV-7/dove/Tn/75
121  AGAGAATGAGGAAAGAGAAG-CAAAGAACA--CATGGCGCTTGGTTTTCCGGATCGCAGT    NDV reference
 53  ATTATTCTCTCTGGGTGATTGCTCT-GATTTCATTGGGTCTGAAGCCAGGT-GAGAGGAT
178  CTTACT-TTTAATGGTAATGACTCTAGCTATCTCCGCAGCTG--CCCTGGTATATAGCAT
111  --CATCAATGAAGTCAATNGATCTATC---CATAATCAACT-TG--TTCCCTTATCGGGG
235  GGGGGCCAGTACGCCGCGTGACCTCGCAGGCAT-ATCGACTGTGATCTCCAAGACAGAGG
163  ATTACTTCCGATATTCAGGCAAAAGTCAGCAGCATATATCGGAGNAACTTGCTAAGTATC
294  ATAAGGTTACGTCTTTA---CTCAGTTCCAAGCA-AGATGTGATAGATAGGATATATAAG
223' CCACTACAACTTGATCAAATCAACNAGGCAATATCATCATCTGCTAGGCAAATTGCTGAT
350  CAGGTGGCCCTTGA--ATCTCCGCT-GGCGCTACTAAACACTGAATCTATAATTATGAAT
283  ACAATCAACTCGTTTCTCGCTCTGAATGGCAGTGGAACTTTTATTTATACAAAATTTATA
407  GCAATAACCTC-TCTCTC-CTATCAA-ATCAACGGGGCTGCGAATAATAGCGGGTCTGGG
343  CA-AATTCACCTGAGTTCG--CAAATGGTTTCAATAGAGCAATGCTTCCCA--ACCCTAA
464  GAGCCTGTTCATGACCCAGATTATATCGGGGGGATAGGCAAAGAACTCATAGTGGACGAC
398  ATCAAAGCTTAAATATGCTAACA-CCTGGT-AATCTAATTGAATTTACTAATTTNATTCC
524  ATTAGTGATGTCACATCATTTTATCCTTCTGCATATCA-AGAACACTTGAATTTCATCCC
456  AACTCCAACAACAAAATNAGGATGTATCAGAATACCATCATTTTCAATGTCATCAAGTCA
563  CGCGCCTACTACAGGATCCGGTTGCACTCGGATACCCTCATTTGACATGAGCACCACTCA
516  CTGGTGTTATACCCATAATATCATTGCTAGTGGATGTCAGGATCATTCAACCAGTAGTGA
643  CTACTGTTATACTCACAATGTGATATTATCTGGTTGCAGAGATCACTCACACTCACATCA
576  ATACATATCGATNGGGGTTGTTGAAGTGACTG-ATCAGGCTNNCCCGAACTTTCGGACAA
703  ATACTTAGCACTTGGTGTGCTTCGGACATCTGCAACAGG-GAGGGTATTCTTTTCTACTC
635  CTCTTTCTATTACATTAGCTGATAATCTAAACAGAAAGTCATGTAGCATTGCAGCAACTG
762  TGCGTTCCATAAATTTAGATGACACCCAAAATCGGAAGTCCTGCAGTGTGAGTGCAACCC
```

Fig 33 (Continuation)

Alignment of the sequenced portion of the APMV-7/dove/Tennessee/75 with NDV HN gene, 49 6% identity in 1215 overlapping

```
695'  GGTTCGGGTGTGATATATTATGTAGTGTTGTCACTGAGACAGAAAATGATGATTATCAAT    APMV-7/dove/Tn/75
822'  CTTTAGGTTGTGATATGCTGTGCTCTAAAGTCACAGAGACTGAGGAAGAGGATTACAAGT    NDV reference
755'  NACNAGAACCGACTCAGATGATCTATGGAAGATTATTTTTTAATGGCACATATTCAGAGA
882"  CAGTTACCCCCACATCAATGGTGCACGGAAGGCTAGGGTTTGACGGTCAATACCATGAGA
815'  TGTCATTGAATGTGAACCAAATGTTCGCAGATTGGGTTGCAAATYATCNAGCAGTTGGAT
942"  AGGACTTGGACACCACAGTCTTATTTAAGGATTGGGTGGCAAATTACCCAGGGGTGGGAG
875'  CAGGAGYAGAGTYAGCAGATTTTGTCATTTTCCCACTCTATGGAGGTGTTÃAAATCACTN
1002" GAGGGTCTTTTATTGACGACCGTGTATGGTTCCCAGTTTACGGAGGGCTCAAACCCAATT
935'  NAACCCTAGGAGCATCTTTAAGCCAGTATTACTATATTCCCAAGGTGCCCACAGTCAATT
1062' CACCCAGTGACACTGCACAAGAAGGGAAATATGTAATATACAAGCGCTATAATAACACAT
995   GCTCTGAGACAGATGCACAACAAAYAGAGAAGGCAAAAGCATCCTATTCACC-ACCTAAA
1122" GCCCCGATGAACAAGATTACCAAATTCGGATGGCTAAGTCTTCATATAAACCTGGGCGAT
1054' GTGCTCCAAATATCTGGGCTCAGGCAGTCGTTAGGTGCAATAAATCTGTCAATCTTGCAA
1182  TTGGTGGAAAGCGCGTACAGCAGGCCATC-TTA---TCTATCAAAGTGTCAACATCTTTG
1114  ATTCATG-TGAAATTCTGACATTNNANACNAGCACTATGA---TGATGGGTGCTGAGGGA
1238' GGTGAGGACCCGGTGCTGACTATTCCACCTAATACAATTACACTCATGGGGGCTGAAGGC
1170' AGACTCGTGATGATAGGAAGAATGCTATACTTTNAATGCGAACGAT
1298  AGAGTTCTCACAGTAGGGACATCTCACTTCTTGTACCAACGAGGGTCTTCATATTTCTCC
```

Fig 34 Sequence of the portion of the APMV-8/Goose/Delaware/76 virus obtained by annealing with a primer to the F gene of the NDV

ACCTAnGCCG CAGGGAAGAC AGTACACGAC ATGCACAATA CTATsACGGT nnCCAGCAAT

CAAtAAAGtg GATsAATCAC TATATgAGGA ATsAAAbAGG ATAACAATsA TgpATCCAAT

TTCCTAATkA TAAAggvksG CTTgAAAGGy bATbGACGaG tcggGGGCGA AAtCTTGCCA

CTbAGTCtGC AGTCAATCTt AGAATCTACA TATTGAACgA TGGGTCAAAb ATCAGTAtAT

CTAATgAgAG CGcgCTATT

Fig 35 Sequence of the portion of the APMV-8/Goose/Delaware/76 virus obtained by annealing with a primer to the HN gene of the NDV

AAAAGsGGnC AATGCACTAC TACAAAATCC TAAGATAGAT GCATTAGTTT CAaAnCTCTA

CTTCACcaCc AGACGTGTTC TATCAAACCT CAGATCAnGn GATAAGGCT AGATCATATAT

nGAnTTGCTA CACTGAGGAC TCGGGAnGT

Fig 36

Sequence of the genomic portion of the APMV-4/Duck/New York/80, 778 nucleotides

GAnATAnnCG AAgcAGAAnn AGGATATnnn CgncnCTacC ATAnCAGGGA GAacAgCTGG
TAAcgacnAC tACCtCAATA ATCCTaAAAA CTGCAGGTAA TACAAaAGGA TcAGCCTATA
GGGAGCTTCA ACAATCGTTA GAAAAaannn cGyAAGCAAC AnTGAGCAAT nCTCcAgcgA
CAATCTCGCC CnTGATCaTC AAGTGCATTC TACCCGnAGC GCATCTtTCC TCACCGTyCG
yaAGGCATAn GTTAynTTAT yTCTGGAGCT AACnGGAGta CCACtnCCCC ACTCAGCAGA
nyTTGnTACG CTAGTCCtAT CCAGACCATG GAACnnAnbA TTGCAGAGAC TCGCCAGAAG
TACtgAGGAT GAAGgAGCTA GGTGCGaACn TCCACGCGAC TCTAGATCAC TCTCGACCAA
TAnnGGCTTT gATCCACCCG GAGACTTtAG CATGGCTAAC TGATCTGTCT ATAGGGGTAT
CTATCTCTAG ATTtAGAGGA ATAGAAnAGa naGnATCTCG CCTGCTCCAT GACAATAGAG
AGAAATTTTG TACACTTGTT TCTCAGATTC ATGAAGGATt GTTCGGTGGT GnaGGAGGGG
TTCGGAATAA TCTGTCACCA GAGTTTGAAA GTTTGCTCAA TGGAACnAAC TTCTGGTTtG
GCGGGAAATA TTCAAACACA AnATTcACTT GGCTTCACAT TAAACAATTG CAGAGACATC
TTATACTCAC AGCGCGTATG AGATCTGGTC AGCACTTACA TCCAATnaaG CATACAGG

Fig 37

Alignment of the sequenced portion of APMV-9/duck/New York/80 with NDV L gene, 50 5% identity in 657 nucleotide overlapping

```
61   TAACGACNACTACCTCAATAATCCTAAAAACTGCAGGTAATACAAAAGGATCAGCCTATA    APMV-9/duck/NY/8C
1                                                AAGTGGCAATGAGAT    NDV reference
121  GGGACCTTCAACAATCGTTAGAAAAAANNNCGYAAGCAACANTGAGCAATNCTCCAGCGA
16   ACAAGGCAAAACAGCTCATGGTAAATAGTACGGGTAGGACA-TG-GCGA-GCTCC--GGT
181  CAATCTCGCCCNTGATCATCAAGTGCATTCTACCCGNAGCGCATCTTTCCTCACCGTYCG
71   CCTGAAAGGGCA-GAGCATC-AGATTATCCTACCAGAGTCACACCTGTCTTCACCATTGG
241  YAAGGCATANGTTAYNTTATYTCTGG-AGCTAACNGGAGTACCACTNCCCCACTCAGCAG
129  TCAAGCACAAACTACTTTATTACTGGAAATTAACTGGGCTACCGCTTCCTGATGAATGTG
300  ANYTTGNTACGCTAGTCCTATCCAGACCATGG-AACNNANA-TTGCA-GAGACTCGCCAG
189  ACTTCGACCACCTCATTCTCAGCAGACAATGGAAAAAAATACTTGAATCGGCCTCTCCTG
357  AAGTACTGAGGATGAAGGAGCTAGGTGCGAACNTCCACGCGACTCTAGATCACTCTCGAC
249  ATACTGAGAGAATGATAAAACTCGGAAGGGCAGTACACCAAACTCTCAACCACAATTCTA
417  CAATANNGGCTTTGATCCACCCGGAGACTTTAGCATGGCTAACTGATCTGTCTATAGGGG
309  GAATAACCGGAGTACTCCACCCCAGGTGTTTAGAAGAACTGGCTAGTATTGAGGTCCCTG
477  TATCTATCTCTAGATTTAGAGGAATAGAANAGANAGNATCTCGCCTGCTCCATGACAATA
369  ATTCAACCAACAAATTTCGGAAGATTGAGAAGA-AGATCCAAATTCACAACACGA-GATA
537  GAGAGAAATTTTGTACACTTGTTTCT-CAGATTCATGAAGGATTGTTCGGTGGTGNAGGA
427  TGGAGAACTGTT-CACAAGGCTGTGTACGCATATAGAGAAGAAACTGCTGGGGTCATCCT
596  GGGGT-TCGGAAT-AATCTGTCACCAGAGTTTGAAAGTTTGCTCAATGGAACNAACTTCT
486  GGTCTAACAATGTCCCCCGGTCAGAGGAGTTCAACAGCATCCGTACGGATCCGGCATTCT
654  GGTTTGGCGGGAAATATTCAAACACAANATTCACTTGGCTTCACATTAAACAATTGCAGA
546  GGTTTCACTCAAAATGGTCCACAGCCAAGTTTGCATGGCTCCATATAAAACAGATCCAGA
714  GACATCTTATACTCACAGCGCGTATGAGATCT--GGTCAGCACTTACATCCAATNAAGCA
606  GGCATCTGATTGTGGCAGCTAGGACAAGGTCTGCGGCCAACAAATTGGTGATGCTAACCC
```

Fig 37 (Continuation)

Alignment of the sequenced portion of APMV-9/duck/New York/80 with NDV L gene, 50 5% identity in 657 nucleotide overlapping

772' TACAGG

666 ATAAGGTAGGCCAAGTCTTTGTCACTCCTGAACTTGTCATTGTGACGCATACGAATGAGA

Fig 38 Sequence of the portion of the APMV-7/Pigeon/Otaru/76 virus obtained by annealing with a primer to the F gene of the NDV

ATTGTCATATCAGATGTATTACcTCCGAGTGATTGGTAAGACTAACATTGTCATGTACAT

CCAAACTGTTATTAAGCGAAbACCGATCCTTCAATTGGCAACACTTGCGTTGACTATTTC

ACACGAGACAGTCTACCACAGAGGCGTGAGAAAAGACTTGTGTTAGAGCCAACACAACCA

TATGTGTCTGCAATAGATTACATTAAATCGGCCAGAGAATTCATCATAAGAGGTGTAAAT

AATbACCTTGGGATGATTGAAGCGGGTTATGACAGTGGATATAACATATTTGACTACCTT

GAGGGAGACTTATCTAAGAAGAtvvATnAGCTGATGGCACGGCGACTTTGTCTCTTAACC

ATTCTGTCTGGTAAGAAGCTGAATCTTCCACtGATTAAGGGGTATGAGCCTATTGTGAAA

TGTAAAA1CCTTACGGAGtTCTTACAGCAAGCATCAGATCAAGGGTTTATTAGtagngnc

ATTGTACAATTGGCAATCACTCCCAAAATTCTGACATACCCAACAAACTTATATTATATG

ATGCGGAAGACTCTgAGCATTATACnATcCaGAGAGGACACCAACCTGATAATnaCCTCT

ATGTACGAAACTGAGGTGTATCAAAGATcAGATTbAGCAATAACATCTAAGGATGCAGTT

GTTGaCCCATTGAATGCTCGCCCTATCTTGATAACAGAGGAGATtATATTTgATCGTTTT

GACTTGCATTcACCTGTGATCTTAAGCAGACATgAGCTAGATCTCCTAGGTCCTCcATGT

TCACAAGTCAGTGATCCCTTATCAAAAbACTTGCATAGACCAATCGGCACTGCATCTACA

AGTtGGTACAAAACGaCCGCACTTCTTGCATGTGATATCCTACGgAggAgggCCCwAGGT

TnaAGCCTTtACCTAtGtGAggGGAGggGGAGhAGwAtGGCTTGTATTGAGGACGCTTTT

CCAGCTGAGACAATTtATTACAATTnATACTTTTCAAATGAAbacnnccCTCCTCAAAGA

AAtATTGGACCnATGCCAACTcaATTCTGTTCnAGCACTGTAtACCAGAACTwAACTGCA

GGCAnaCCCtGTCTCTCGGGTTnGTTnAACAATtCCAAGtACtAtGGAAtgAGAATTCtG

AtGAAACnGAtATCTCATCGATnAGCnGnGnGAATTTCAtAnGTCAAnpGGtACCTCTCA

ATTnAtGCCACTtACTTCAtGCTGATCTAGATCtACnAGTTsAGaCdCCCAGAGnGGTTn

GGnACTCanGCTtaACsAAsAgATTCATgATgGCCACAAAnGgGTsAAAAAgAGGnGGAC

ATTsGGTAAT bAAGnbAbAb GGnAGbGAbG AGnnGCnGCT bAG

Fig 39 Alignment between the APMV-?/Pigeon/Otaru/76 (Pigeon/Otaru) and the L gene of NDV

```
=====================================================================
HUSAR FASTN Program,   1358 bases versus EMBL viral library
=====================================================================
The best score is with NDV L gene, 49 3% identity
---------------------------------------------------------------------

   1'                                   ATTGTCATATCAGATGTATTACCT  <--- Pigeon/Otaru

4441' CTTATTTGAATATGCAGCACTTGAAGTGCTCCTCGACTGTTCTTACCAACTCTATTATCT  <--- NDV (L gene)

  25' CCGAGTGATTGG-TAAGACTAACATTGTCATGTACAT-CCAAACTGTTATTAAGCGAAAC

4501' GAGAGTAAGAGGCCTAGAC-AATATTGTCTTATATATGGGTGATTTATACAAGAATATGC

  83' CGATCCTTC-AATTGGCAACACTTGCGTTGACTATTTCACA-CGAGACAGTCTACCACAG

4560" CAGGAATTCTACTTTCCAACA-TTGCAGCTACAATATCTCATCCCGTCATTCATTCAAGG

 141' AGGCGTGAGAAAAGACTTGTGTTAGAGCCAACACAACCATATGTGTCTGCAATAGATTAC

4619" TTACATGCAGTGGGCCTGGTCAACCATAACGGATCACACCAACTTGCAGATACGGATTTT

 201' ATTAAATCGGCCAGAGAATTCATCATA-AGAGGTGT-AAATAATNACCTTGGGATGAT-T

4679" ATCGAAATGTCTGCAAAACTGTTAGTATCTTGCACTCGACGTGTGATCTCCGGCTTATAT

 258' GAAGCGGGTTATGACAGTGGATATAACATATTTGACTACCTTGAGGGAGACTTATCTAAG

4739" TCAGGGAATAAGTATGATCTGCTGTTCCCATCTGTCT---TAGATGATAACCTGAATGAG

 318' AAGATNNATNAGCTGATGGCACGGCGACTTTGTCTCTTAACCATTCTGTCTGGT--AAGA

4796" AAGATGCTTCAGCTGATATCCCGGTTATGCTGTCTGTACACGGTACTCTTTGCTACAACA

 376' AGCTGAATCTTCCACTGATTA-AGGGGTATGAGCCTATTGTGAAATGTAAAATCCTTACG

4856' AGAGAAATC--CCGAAAATAAGAGGCTTATCTGCAGA-AGAGAAATGTTCAGTACTTACT

 435' GAGTTCTTACAGCAAGCATCAGATCAAGGGTTTATTAGTAGNGNCATTGTACAATTGGCA

4913' GAGTATCTACTGTCGGATGCTGTGAAACCATTACTTAGCCCTGATCAGGTGAGCTCTATC

 495' ATCACTCCCAAAATTCTGACATACCCAACAAACTTATATTATATGATGCGGAAGACTCTG

4973  ATGTCTCCTAACATAATTACATTCCCAGCTAATCTGTACTACATGTCTCGGAAGAGCCTC

 555' AGCATTATACNATCCAGAGAGGACACCAACCTGATAATNACCTCTATGTACGAAACTGAG

5033' AATTTGATCAGGGAAAGGGAGGACAAGGATTCTATCCTGGCGTTGTTGTTCCCCCAAGAG
```

Fig 39 Continuation

615' -GTGTATCAAAGATCAGATTAGCAATAACATCTAAG-GATGCAGTTGTTGACCCATTGAA

5093 CCATTATTAGAGTTC-CCTTCTGTGCAAGATATTGGTGCTCGAGTGAAAGATCCATTCAC

673' TGCTCGCCCTATCTTGATAACAGAGGAGATTATATTTGATCGTTTTGACTTGCATTCAC-

5152 CCGACAACCTGCGGCATTTTTGCAAGAG-TTAGATTTGAGTGCTCCAGCAAGGTATGACG

732' CTGTGA-TCTTAAGCAGACATGAGCTAGATCTCCTAGGTCCTCCATGTTCACAAGTCAGT

5211 CATTCACACTTAGTCAGA-TTCATCCTGAGCTCACA---TCACCA-AATCCGGAGGAAG-

791' GATCCCTTATCAAAAACTTGCATAGACCAATCGGCACTGCATCTACAAGTTGGTACAAAA

5265' ACTACTTAGTACGATACTTGTTCAGAGGAATAGGGACTGCATCCTCCTCTTGGTATAAGG

851' CGACCGCACTTCTTGCATGTGATATCCTACGGAGGAGGGCCCNAGGTTNAAGCCTT-TAC

5325 CATCCCATCTCCTTTC-TGTACCCGAGGTAAGATGTGCAAGACACGGGAACTCCTTATAC

910' CTATGTGAGGGGAGGGGGAGNAGNATGGCTTGTATTGAGGACGCTTTTCCAGCTGAGACA

5384 TTAGCTGAAGGAAGCGGAGCCATCATGAGTCTTCTCGAACTGCATGTACCACATGAAACT

970' ATTTATTACAATTNATACTTTTCAAATGA-AACNNCCCTCCTCAAAGAAATATTGGACCN

5444 ATCTATTACAATACGCTCTTTTCAAATGAGATGAACCCCCCGCAGCGACATTTCGGGCCG

1029' ATGCCAACTCAATTCTGTTCNAGCACTGTATACCAGAACTNAACTGCAGGCANACCCTGC

5504 ACCCCAACCCAGTTTTTGAATTCGGTTGTTTATAGGAACCTACAGGCGGAGGTAACATGC

1089' TCTCTCGGGTTNGTTNAACAATTCCAAGTACTATGGAATGAGAATTCTGATGAAACNGAT

5564' AAGGATGGATTTGTCCAAGAGTTCCGTCCACTATGGAGAGAAAATACAGAGGAAAGCGAC

1149' ATCTCATC-GAT-NAGCNGNGNGAATTTCATANGTCAANNGGTACCTCTCAATTNATGCC

5624 CTGACCTCAGATAAAGCAGTGGG-GTATATTACATCTGCAGTGCCCTACAGATCTGTATC

1207' ACTTACTTCATGCTGATCTAGATCTACNAGTTNAGACCCCAGAGNGGTTNGGNACTCANG

5683 A-TTGCTGCATTGTGACATTGAAATCCCTCCAGGGTCCAATCAAAGCTTACTAGATCAA-

1267' CTTAACNAAAGATTCA----TGATGGCCACAAANGGGTNAAAAAGAGGNGGACATTNGGT

5741' -TTAGCTATCAATTTATCTCTGATTGCCATGCATTCCGTAAGGGAGGGCGGGGTAGTGAT

1323' AATNAAGNNANANGGNAGNGANGAGNNGCNGCTNAG

5800 CATCAA-AGTGTTGTATGCAATGGGATACTACTTTCATCTACTCATGAACTTGTTCGCTC