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**Studies on the Ecology of Animal
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ABSTRACT

Before 1979 the situation concerning animal influenza in Israel was completely obscure: either research, or serological surveillance, or routine diagnostics and epizootiological service on animal influenza did not exist. Award of the present project by BARD foundation resulted in the establishment of a special unit in the Kimron Veterinary Institute responsible for the studies on the ecology of animal influenza. During the research the specimens from more than 2700 birds and 800 mammalian animals were examined. Influenza viruses have been isolated from all kinds of poultry raised in Israel (turkeys, chickens and ducks) as well as from various species of feral, especially, migrating birds (starlings, mallard and pintail ducks, coots, and rock partridges). A total of 56 influenza virus isolations have been identified including 26 isolations from feral birds and 30 isolations from poultry. Besides, three unidentified hemagglutinating (HA) agents isolated from turkeys in the past (1971, 1973 and 1978) were identified retrospectively as influenza viruses. Seven serologically different influenza virus antigenic combinations have been identified. One of the studied cases has offered evidence suggesting immediate epizootiological connection between occurrence of influenza among the mallard ducks and influenza outbreak in a turkey farm. The recent influenza outbreaks in turkey farms in North Israel from which a strain of H1N2 antigenic combination was isolated has displayed a tool for examining possible connections between avian and mammalian (swine and human) influenza. In the course of the surveillance more than 50 isolates were identified as avian paramyxoviruses which was important although accessory result of the studies.

INTRODUCTION

Israel is a unique place for the studies on the ecology of animal influenza viruses because of its geographical, ecological and economical peculiarities. First, Israel is located along the main flyway of feral birds migrating from Eastern Europe (mainly European part of USSR) to North-East Africa. Secondly, Israel has a highly developed poultry industry which is run throughout the country in farm villages of two kinds: moshavs with individual poultry farms containing 1,000 to 5,000 birds and kibbutzes which are communal farms with large poultry houses containing tens of thousands of birds. The third peculiarity is a relative isolation of Israel from neighbouring countries resulting in the absence of any poultry trade between them; this situation presents artificial conditions for a natural experiment on elucidation of the possibility of interspecies transmission of influenza viruses from wild birds to poultry.

Before 1979 the situation concerning animal influenza in Israel was completely obscure: either research or serological surveillance, or routine diagnostics and epizootiological service on animal influenza did not exist. The adequate studies initiated in 1978 were considerably intensified since autumn 1979 when a project "Studies on the ecology of animal influenza viruses in Israel" was awarded by a grant from US-Israel

Binational Agriculture Research and Development Fund (BARD). This permitted to establish a special unit in the Kimron Veterinary Institute responsible for the performance of the project. The studies were carried out in collaboration with the Veterinary Service of Israel Ministry of Agriculture (including local private veterinary practitioners), Israel Ministry of Health (The Chief-Scientist Office); Department of Zoology of Tel-Aviv University, Israel Committee of Natural Reservations, Israel Association for the Defence of Natural Environments and Israel Hunter Association.

MATERIALS AND METHODS

Cloacal and tracheal swabs were used as the main field source for virus isolation. Sometimes, in the case of domestic birds, organ materials such as trachea, lungs and brain were used. The swabs were placed into support medium (PBS pH 7.2 containing 50% glycerol with penicillin, streptomycin and fungizone) and kept in dry ice while being transported to the laboratory. A 0.2 ml vol. of each sample was inoculated into allantoic cavity of 10- or 11-day-old chick embryonated eggs, three eggs being used for each sample. The eggs were incubated at 37° C up to the death of embryos or till six days p.i. if the embryos remained alive. The allantoic fluids were tested individually for hemagglutination (HA) activity. HA agents propagatable in embryonated eggs after Millipore filtration were used in the studies. Those HA agents which were positive in neuraminidase (Nase) reaction test and negative in HA inhibition (HI) test with anti-Newcastle disease virus (NDV) serum were taken for

further identification as influenza viruses. The identification was carried out using a set of monospecific goat antisera against all the influenza virus HA and NA antigenic subtypes provided by the Cooperating Investigator Prof. R. G. Webster (WHO Collaborating Center for Ecology of Influenza Viruses in Animals, St. Jude Children's Research Hospital, Memphis, Tennessee, USA) as well as a set of reference complete antisera provided by Dr. J. J. Skehel (World Influenza Centre, National Institute for Medical Research, Mill Hill, London).

HA titration and HI test were performed in microtitre plates with receptor-destroying enzyme (RDE) treated sera (Palmer et al., 1975).

NA activity was determined by thiobarbituric acid assay (Warren, 1959) in modification of Aminoff (1961). NA titration and NI test were performed either as described in 'Advanced Laboratory Techniques for Influenza Diagnosis (1975) or by microtechnique modification used in World Influenza Centre.

Double immunodiffusion test (DID) was done in 1.5% agarose dissolved in phosphate-buffered saline (pH 7.2) containing 0.1% sarcosyl NL₉₇ and 0.1% sodium azide using either "Hyland" plastic plates, or preformed gel plates ("Mely" Springfield, Virginia, USA). Preparation of the antigens from the isolates and the test procedure were carried out according to the "Advanced Laboratory Technique for Influenza Diagnosis"

(Palmer et al., 1975).

RESULTS

I. Isolation of influenza viruses from poultry

1) Isolation of influenza virus from a turkey farm in moshav Ramon (Jezrael Valley) in February 1979. The outbreak of a respiratory disease occurred in a breeder farm. The disease was characterized by mild respiratory syndrome and sharp drop in egg production with mortality about 20%. Cloacal and tracheal swabs taken from sick birds were inoculated into embryonated eggs. One HA agent was isolated from a cloacal swab and identified as A/turkey/Ramon, Israel/79 (H7N2) influenza virus.

2) Isolation of influenza virus from a chicken farm in kibbutz Degania (Lake Kinneret) in June 1980. A respiratory disease affected the chickens of the age of 6 weeks and characterized by rales, tracheitis, pneumonia, nephritis and poor weight gain. Morbidity was about 50 per cent and mortality was about 10 per cent. From a brain specimen a HA agent was isolated and identified as A/chicken/Degania, Israel/80/H7N2) influenza virus.

3) Isolation of influenza virus from a duck farm in kibbutz Gan Shmuel in December 1980. Since autumn 1980 symptoms of an upper respiratory disease were observed in ducklings. In December 1980 the disease spread to breeding flock and was

characterized by rales and sinusitis though morbidity was about 10 per cent. From a cloacal swab a HA agent was isolated and identified as A/duck/Gan Shmuel, Israel/80 (H11N2) influenza virus.

4) Isolation of influenza virus from a turkey farm in moshav Hatsav in January 1981. The outbreak of a respiratory disease having affected 4 private turkey farms was characterized by rales, sinusitis, nasal discharge, pneumonia, air succulitis and pericarditis. Morbidity was 100 per cent, while mortality was about 10%. From a mixture of various organs taken from sick birds a HA agent which was isolated and identified as A/turkey/Hatsav, Israel/842/80 (H11N2). The outbreak then continued to proceed, but during the spring 1981 new isolations of influenza virus were not picked up, while there was a number of the isolates of NDV and Yucaipa-like paramyxoviruses. Therefore, a mixed infection of influenza, Newcastle disease and Yucaipa-like paramyxovirus infection is evident. The interesting fact was that NDV and Yucaipa viruses displaced and supplanted influenza virus from this local turkey reservoir. This was supported by serological surveillance.

5) Isolation of influenza viruses from an outbreak of respiratory disease in Lower Galilee, in April - May 1982. The outbreak burst in a number of turkey farms in three neighbouring villages: Beit Lehem Glilit, Havat Mekorot and Nahalal. The disease was characterized by rales, sinusitis, and affection of reproductive tract. Morbidity reached 70 - 95 per cent and morta-

lity fluctuated in the limits of 40 - 50 per cent. Promptly performed specific investigation resulted in isolation of 25 HA agents identified as A/turkey/Galilee, Israel/82 (H1N2). Serological surveillance provided solid support for the causative connection between the influenza virus isolates and the outbreaks. The H1N2 antigenic combination has revealed a valuable tool for the elucidation whether the H and N antigenic components originated from either avian or mammalian reservoirs.

The data on characterization of influenza virus outbreaks in poultry farms in Israel are presented in the Table 1.

II. Isolation of influenza viruses from feral birds

More than 1500 feral birds including alive ones, which were trapped, and dead ones, which were picked up were investigated. Cloacal and tracheal swabs served as the main source of the material from which the viruses were isolated. The investigated birds included more than 20 avian species (Tab. 2). Influenza viruses were isolated from starlings (1978), mallard ducks (1979), pintail ducks (1980), coots (1980 and 1983) and rock partridges (1981).

Starlings (*Sturnus vulgaris*) were chosen as an object of prior interest because of the remarkable ecological peculiarities of this species. The starlings wintering in Israel came from the European part of USSR (Dement'ev, 1970, Yomtov, 1980). In the country of nesting they are rather homogeneously dispersed in wide territories while in Israel on the background of diffuse

distribution most of them are concentrated in 5 particular spots in which the birds spend nights amassing in millions in small areas covered by bushes or trees, the closeness reaching 530 birds per cubic meter (Yomtov, 1982). During the day the birds spread all over the country invading farm-yards in search of food. One such area of roosting (Hamat Gader near the Southern edge of Lake Kinneret) was chosen for investigation and recently dead birds were used for swabbing. One isolated HA agent was identified as A/starling/Kinneret, Israel/78 (H7N7) influenza virus.

Mallards (*Anas platyrhynchos*) winter in Israel throughout the country in water reservoirs and humid fields. At the beginning of January 1979 about 200 birds were found dead near the village Ramon. Fifteen of them were frozen and brought to the Kimron Veterinary Institute for post-mortem investigation. The opportunity being used, the cloacal and tracheal swabs were taken from the birds and from all of them HA agents were isolated and identified as A/mallard/Ramon, Israel/79 (H7N2).

Pintails (*Anas acuta*) have the same ecological characteristics as mallards. One HA agent isolated from them was identified as influenza virus, its N₂ belonging to N4 antigenic subtype. Its HA, however, definitely differs from each known influenza virus antigenic subtypes. Further research using more advanced techniques is needed for the decisive evidence that its HA antigen belongs to a novel antigenic subtype.

Rock partridges (*Alectoris graeca*) are resident birds distributed throughout Israel. The investigation was carried out during the hunting period (September to January, 1980-81) with the kind help of Israel Hunter Association. A total of 126 carcasses were included in the studies. Cloacal and tracheal swabs and, in some cases, blood samples were taken from the carcasses either immediately or within an hour after shooting. The HA agents were isolated from two birds shot near the village Beit Nir, one from a cloacal swab (7c) and the other from a tracheal swab (9t). Both the HA agents were identified, respectively, as A/rock partridge/Beit Nir, Israel/7c (H7N2) and A/rock partridge/Beit Nir, Israel/9t (H7N2). Thus, both the influenza virus isolates have the same HA-Nase antigenic combination as the isolates from mallards, turkeys, and chickens. This makes a basis for the assumption that H7N2 combination is widely spread in different avian species. This is, however, the first isolation of influenza virus from this species.

Coots (*Fulica atra*) a migrating waterfowl species not belonging to the duck family (Anatidae) also is widely spread in the country. In 1980 from trapped birds 4 HA agents were isolated and identified as A/coot/Shluhot, Israel/80 (H10N4). Among these four isolates there were (in some cases prominent) differences in the HA antigenicity, which can be attributed to different avidity of the HA antigens.

Just recently (January, 1983) two HA agents were isolated from coots trapped in the same place (village Shluhot). This time the HA-Nase antigenicity of the isolate was identified as A/coot/Shluhot, Israel/83 (H10N2) influenza virus. Thus the N4 antigen of the influenza viruses previously isolated at the same place appeared to be changed for the N2 antigen prevailing among the strains isolated in Israel (Tab. 2).

III. Retrospective identification as influenza viruses of HA agents isolated in Israel in the past

This line of work was an integral part of the project since the knowledge about the influenza viruses circulated in the past is of the great importance for ecological surveillance and, thus, for reconstruction of possible epizootiological connections. The best way to get such knowledge was to examine any isolations of unidentified HA agents made in the past which would be fortunately preserved in a viable form up to now. Three such HA agents isolated in Israel in 1971, 1973 and 1978 were succeeded in being identified as influenza viruses.

In spring 1971 an HA agent was isolated at a turkey farm in kibbutz Kfar Vitkin (Coastal Plain) from brain specimens of birds suffering from a respiratory syndrome accompanied by torticollis. Although morbidity was high, mortality was quite low. The isolated HA agent was inhibited by neither anti-NDV nor anti-fowl plague virus antisera and was stored in the

laboratory of Dr. Y. Akov (Institute for Biological Research, Ness Ziona). In 1980 the isolate was re-passaged and identified as A/turkey/Kfar Vitkin, Israel/71 (H5N2) influenza virus.

In 1973 an outbreak of a respiratory disease occurred in a turkey farm in the village Ramon in which in 1979 (see above) the H7N2 influenza viruses were isolated from wild mallard ducks and from turkeys at the same farm. An isolated HA agent being not NDV was not identified further and was stored in the laboratory of Dr. K. Hornstein (Department of Vaccine Development, Kimron Veterinary Institute). In 1981 the HA agent was identified as A/turkey/Ramon, Israel/73 (H5N2) influenza virus. Thus, the HA-Nase antigenic combination was different from H7N2 viruses isolated at the same place in 1979 although both share the same N2 Nase antigenic subtype.

In August 1978 an outbreak of respiratory disease occurred in the kibbutz Gan Shmuel and was characterized by rales, pneumonia, conjunctivitis, sinusitis and diarrhea. The morbidity reached 90 per cent and mortality was about 30 per cent. An unidentified HA agent isolated from a tracheal swab was stored until retrospective identification in 1981 as A/turkey/Gan Shmuel, Israel/78 (H5N2).

Thus, all the 3 retrospectively identified influenza virus isolates turned out to have the same H5N2 antigenic combination. From these results an assumption can be preposed that H5N2 combination prevailed in poultry farms in Israel at least during 1971-78 period and in 1979 it was replaced by the recently pre-

vailing H7N2 combination.

The described data are presented at the Tab. 2.

IV. Surveillance of mammalian species with respect to influenza

The studies on the mammalian species concerned horses and pigs. During the period under consideration there was no serious outbreaks of respiratory disease in horse herds and pig farms. Therefore, the research was performed as serological surveillance in horse herd in Acco and in two pig slaughter houses: (a) in Nasareth to where the pigs are brought from arabic villages located around in the countryside and (b) in a special piggery in kibbutz Lahav raising the pigs for research purposes. Meanwhile, no antibody against influenza viruses were found in 18 horse sera collected. As to pigs, no virus was isolated and about 1000 specimens of blood were accumulated for performance of HA inhibition test which is intended to be carried out in the not-too-far-distant future.

V. Isolation of avian paramyxoviruses during the surveillance of feral birds, poultry and pig husbandry.

During the wide surveillance of both feral birds and poultry carried out in the limits of the present project a number of paramyxovirus (PMV) strains were isolated.

In the winter 1978-79 a wave of outbreaks of a respiratory disease occurred and spread through a number of turkey farms in different parts in Israel. The disease was characterized by

symptoms which ranged from mild rales to severe respiratory distress including nasal discharge, sinusitis, conjunctivitis and pneumonia. Low egg production was observed in breeder farms. Morbidity was high, reaching 100 per cent, while mortality fluctuated within the wide range of 5 to 90 per cent. That time avian PMV strains belonging to the second PMV (PMV-2 "Yucaipa-like") group (Alexander, 1961) were isolated from turkeys. Since then more than 50 isolates were picked up from poultry (turkeys and chickens) as well as from feral birds (mallard ducks, cattle egrets and coots). Besides, NDV (PMV-1 group, Alexander 1981) strain was isolated from starlings wintering in kibbutz "Neve Eitan". Besides, a HA agent identified as a PMV strain belonging to the 3d group (PMV-3, Alexander, 1981) was isolated from pigs.

The list of the PMV strains isolated in Israel during the 1979-1981 period is presented in the Tab. 4.

As it was described above, Yucaipa-like viruses spread all over the country can interfere with influenza outbreak, this raising peculiar situations the clinico-pathological, immunological and epizootiological points of view.

Quite unexpected finding was an isolation of avian PMV belonging to the group 3 (PMV-3) from pigs.

DISCUSSION

Thus, the studies on the ecology of animal influenza viruses in Israel have shown that influenza viruses circulate in avian populations, including both feral birds and all kinds of poultry raised in Israel.

During the period of the research specimens from more than 2700 birds - domestic and feral - and from more than 800 mammalian animals (pigs, horses, wild-boars and hares) were examined. This number included more than 1700 domestic birds: turkeys (1540), chickens (60), and ducks (110) and 1065 feral birds: mallard, teal and pintail ducks (85), coots (110), moorhens (20), starlings (310), rock partridges (130), cattle egrets (120), pigeons and turtle doves (140); quails (40), larks (20) and some other various species of migrating birds (90).

A total of 55 isolations of HA agents have been identified as influenza viruses. From them 30 isolations were derived from poultry: turkeys - 27, chickens 1, ducks - 2, and 25 isolates were derived from feral birds: starlings - 1, mallard ducks - 16, pintail ducks - 1, coots - 5, and rock partridges - 2.

In addition to the viruses isolated within the last three-year period, a number of unidentified HA agents isolated in Israel in the past (by veterinarians dealing with Newcastle disease virus) and fortunately preserved in a viable form up to now were included into the studies. From this source 3 HA agents isolated from turkeys in 1971, 1973 and 1978

were identified retroactively as influenza viruses.

Seven serologically different influenza A virus HA-Nase antigenic combinations have been identified: H7N7, H7N2, H5N2, H10N4, H11N3, H1N2, and H10N2. Of these combinations, H5N2 was found in all the three retroactively identified isolates from turkeys and was not found in the recent isolations. H7N2 combination being more frequent was isolated from turkeys, chickens, mallard ducks and rock partridges. H10N4 combination was isolated from coots and H11N3 was isolated from domestic ducks and turkeys. H10N2 combination has been just recently isolated from coots. H7N7 combination being isolated from starlings was serologically identical to A/FPV/Dutch/27 (H7N7) prototype of classic fowl plague virus by both HA and Nase antigens, but the isolate appeared to be avirulent. The antigenic combination H1N2 was isolated from turkeys just recently (May, 1982) from the outbreaks of respiratory disease in breeder flocks in Lower Galilee (Beit Lehem Glilit, Nahalal and Havat Mekorot). The HA of an isolate from a pintail duck being different from any known HA antigenic subtype has not yet been identified (Tab. 3).

All the outbreaks of influenza registered in poultry farms, including those "old" outbreaks from which the unidentified HA agents were isolated and were identified as influenza virus retroactively were characterized by mild respiratory syndrome with low to moderate mortality. The outbreaks in breeder turkey farms in moshavs Ramon, Beit Lehem Glilit, Nahalal and Havat Mekorot were characterized by sharp drop in egg production

and by some other symptoms of affection of reproductive tract. There was evidence on considerable influence of concomitant infections on severity of the disease, this influence being confirmed by experiments on artificial infection of turkeys in laboratory conditions. As to antigenic composition of the isolates from poultry, it is of interest that all of them isolated within 1971 - 1983 period had the same N2 subtype of N₂ which was combined in consecutive order with H5, H7, H11 and H10 subtypes of HA.

The influenza viruses from coots (non-duck waterfowl), starlings and rock partridges were isolated for the first time. The isolation of influenza viruses from non-waterfowl birds is of particular interest. The point is that the significance of wild ducks as carriers of influenza viruses was investigated thoroughly, including both ecological and virological aspects (Slemons et al., 1974; Webster et al., 1976; 1978; Laver and Webster, 1979; Kida and Yanagawa, 1979; Kocan et al., 1980; Hinshaw et al., 1980; Sriram et al., 1980) while the corresponding knowledge concerning non-waterfowl birds is rather scanty. Starlings, wintering in Israel, offer a remarkable opportunity for the research. They migrate to Israel from the vast area of European part of USSR, congregating in millions in some particular places for night spending (Yomtov et al., 1977; Yomtov, 1980). In the daytime, the birds spread all over the countryside searching for food and invading into farm yards. Up to now, an H7N1 influenza virus strain and a lentogenic strain of NDV were isolated from the starlings. Thus, the ecological peculiari-

ties of this species in Israel make it to be of especial interest for investigation of non-waterfowl birds as a potential reservoir for circulation of influenza viruses.

Analysis of the isolate antigenicity, chronology of the isolations and their distribution in the area permitted to make some tentative conclusions about possible epizootiological connections. It can be suggested that H5N2 strains circulated in poultry in Israel during at least 8 years and then were replaced by H7N2 and H11N2 strains. Among the influenza outbreaks registered in poultry in Israel, there are two cases when the outbreaks occurred twice in the same place: (a) moshav Ramon in which the first outbreak was in 1973 and the second one in 1979, both having occurred in the same turkey farms and (b) kibbutz Gan Shmuel in which the first outbreak was observed in 1978 in the turkey farm and the second one in 1980 in the duck farm. In both cases, the first outbreak was caused by H5N2 strain, while the second one was caused by H7N2 strain in Ramon and by H11N2 strain in Gan Shmuel. In Ramon, the H7N2 strain was isolated also from migrating mallard ducks found dead in fields located in about one kilometer from the turkey farms in which a month later the H7N2 strain-caused influenza outbreak burst out. Such ecological circumstances offer strong evidence on interspecies transfer of influenza virus from wild migrating ducks to domestic turkeys. However, antigenic similarity between the isolates from mallards and turkeys shown by serological methods using polyclonal sera is insufficient for the conclusion about the identity of the two strains, and hence, about the

interspecies transfer of the influenza virus strains. Advanced analysis of influenza virus antigenic determinants using monoclonal antibodies, as well as RNA segment analysis of both strains, is needed for the decisive conclusion (Hinshaw et al., 1980; Sriram et al., 1980), these studies being under initiation.

The recent isolation of influenza virus of H1N2 antigenic combination from turkeys is a fact raising a question of high ecological significance: what is the origin of this recombinant strain? Namely, the question is whether this recombination originated primarily in swine or human population and then it transferred to turkeys, or the recombination occurred primarily inside the turkey (or, generally, avian) reservoir (with potential capacity to transfer to mammalian reservoir). In connection with this a particular question is the origin of N2 of the H1N2 isolate, namely whether it is of the "early" ("Singapore-like") type, or of the "late" ("Hong-Kong-like") type (Kendal and Kiley, 1973). Up to now all the studied N2 Nases found in the strains isolated from birds within the period of 1965 - 1981 were of the "early" type (Webster et al., 1982) demonstrating remarkable stability over many years contrary to the N2-containing strains of mammalian origin. If N2 of H1N2 turkey isolate turns out to be that of the "early" type it would show rather avian source of the isolate origin. Anyhow, elucidation of the origin of the turkey H1N2 isolate would be a tool for examining of postulated connections between the avian and mammalian reservoirs for influenza viruses. However, the examination will need the application of the modern technical approaches concerning gene analysis

of the isolate RNA and monoclonal antibody-mediated analysis of their antigens. Similar characterization of the other native strains isolated in Israel will be the main purpose of the future continuation of the project.

The isolation and identification of avian paramyxoviruses (PMV) in Israel was important although accessory result of the studies on the project. In fact, it was the first isolation of avian PMV (except NDV) in Israel. A connection of this virus with a wave of the outbreaks at a respiratory disease which was spread all over the country has been established. The Yucaipa-like PMV-2 was isolated from turkeys (predominantly) and chickens as well as from feral birds: mallard ducks (1979), cattle egrets (1980, 1981) and coots (1981). The studies on the characterization of the native isolates have shown some biological and antigenic peculiarities of them as compared with the reference strains. Clinico-pathological peculiarities of the disease due to the PMV itself as well as to the mixed infection with other infectious agents were described. Some epizootiological connections were established. Isolation of PMV-3 from pigs reveals unprecedented possibility for suggestion about transfer of avian paramyxoviruses from avian to mammalian reservoir.

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Table 1. Characterization of influenza outbreaks in poultry farms in Israel

Place	Date of outbreak	Species	Clinico-pathological description	Morbidity	Mortality	Source of isolation	Isolate antigenic subtype
RAMON (moshav)	Mar. 1979	Turkey (breeders)	Rales, sinusitis, nasal discharge, diarrhea, prolapsus of urogenital and intestinal tracts; Sharp drop in egg production, soft egg shells, shells without content.	80%	20%	cloacal swab	H7N2
DEGANIA (kibbutz)	Jul. 1980	Chicken	Rales, pneumonia, tracheitis, nephritis, poor weight gain.	50%	10%	Brain	H7N2
GAN SHMUEL (kibbutz)	Dec. 1980	Duck	Mild sinusitis, conjunctivitis	30%	2%	Cloacal swab	H11N2
HATZAV (moshav)	Dec. 1980	Turkey	Rales, sinusitis, nasal discharge	90%	10%	liver and lungs combined homogenate; cloacal & tracheal swabs	H11N2
BEIT LEHEM GLILIT	May 1982	Turkey (breeders)	Rales, nasal discharge, sharp drop in egg production up to 0, emergence of eggs inside the abdominal cavity with subsequent peritonitis and sudden deaths.	95%	50%	tracheal and cloacal swabs	H1N2
NAHALAL (moshav)	--	--	--	80%	40%	--	H1N2
HAVAT MEKOROT (moshav)	--	--	--	70%	40%	--	H1N2

Table 2. Characterization of influenza outbreaks occurred in the past with retrospective identification of the viruses

Place	Date of outbreak	Species	Clinico-pathological description	Morbidity	Mortality	Source of isolation	Isolate antigenic subtype
KFAR VITKIN (kibbutz)	Jun. 1971	Turkey	Rales, sinusitis, torticollis.	90%	5%	Brain	H5N2
RAMON (moshav)	Jun. 1973	Turkey	Rales, pneumonia	no data	no data	Lungs	H5N2
GAN SHMUEL (kibbutz)	Aug. 1978	Turkey	Conjunctivitis, sinusitis, pneumonia, diarrhea	90%	30%	Trachea	H5N2

Table 3. List of influenza virus strains isolated in Israel

Strain	Year of Isolation	Year of Identification	Number of Isolate
1. A/turkey/Kfar Vitkin/H5N2	1971	1980	1
2. A/turkey/Ramon/H5N2	1973	1980	1
3. A/turkey/Gan Shmuel/H5N2	1978	1980	1
4. A/starling/Kinneret/H7N7	1978	1979	1
5. A/mallard/Ramon/H7N2	1979	1979	16
6. A/turkey/Ramon/H7N2	1979	1979	1
7. A/chicken/Degania/H7N2	1980	1980	1
8. A/coot/Shluhot/H10N4	1980	1980	4
9. A/pintail duck/Shluhot/H?N4	1980	1980	1
10. A/rock partridge/Beit Nir/H7N2	1981	1981	2
11. A/duck/Gan Shmuel/H11N2	1980	1981	2
12. A/turkey/Hatzav/H11N2	1980-81	1981	2
13. A/turkey/Beit Lehem Glilit/H1N2	1982	1982	9
14. A/turkey/Nahalal/H1N2	1982	1982	12
15. A/turkey/Havat Mekorot/H1N2	1982	1982	4
16. A/coot/Shluhot/H10N2	1983	1983	2

EVALUATION OF THE RESEARCH ACHIEVEMENTS WITH RESPECT TO THE
ORIGINAL RESEARCH PROPOSAL AND ITS OBJECTIVES

All the research achievements are in accordance with original proposal and its objectives, the latter being completely fulfilled. Some of the findings, namely, isolation and identification of plenty of avian paramyxovirus strains from poultry and feral birds as well as from a mammalian species (swine) is a valuable addition to the clinico-pathological description of a respiratory disease caused by these viruses and to understanding of ecological situation in the area which is the main point of the project.

DESCRIPTION AND RESULTS OF COOPERATION

The cooperative institution in the U.S.A. is World Health Organization Collaborating Center for Ecology of Influenza Viruses in Animals which is affiliated with the Division of Virology of St. Jude Children's Research Hospital, Memphis, Tennessee, U.S.A. The collaborator, Prof. Robert G. Webster, Director of this Centre, initiated the establishment of an International Network for Surveillance of Influenza Infections among Domestic and Wild Species of Animals, this network including 8 international laboratories throughout the world and 21 laboratories in the U.S.A.. Therefore such an institution is in a position to give advice on the project and to provide the specific strains and reagents for the characterization

of influenza virus. However, the formal prohibition for sending newly isolated influenza virus strains to the U.S.A. from abroad makes more close kind of cooperation hardly possible. Therefore, apart from all the field work which is evidently can be done only in Israel, almost all the laboratory and experimental work has to be done also in Israel. Therefore, the cooperative research involved active collaboration in the following forms:

1. Joint planning of research, evaluation of results and current consultations by correspondence.
2. Provision of specific reagents.
3. Mutual visits to each other laboratories, evaluation of the results, discussions, and mastering of modern techniques.
4. Performance of some joint experiments in U.S.A. with certain non-infectious viral components isolated from the native isolates.

Visits

1. In November 1980 Prof. R. Webster, Cooperating Investigator, visited Kimron Veterinary Institute. The following purposes were fulfilled during his visit : a) inspection of laboratory design for isolation and identification of native influenza virus strains; b) joint experiments proving the antigenic characterization of the native strains; c) visit of some poultry farms; d) participation in the field surveillance: cooperation with hunter group and swabbing of shot rock

partridges; observation on waterfowl in the "Emeq Hula" reservation; observation on the place of night spending of migrating starlings in Hamat Gader; e) comprehensive discussions on the current work and planning the further studies.

2. In April - May 1981 the Principal Investigator visited the Cooperating Institution in Memphis, Tennessee. The aim was to study modern techniques for antigenic and genetic analysis of influenza viruses. The official prohibition to bring any viral strains into U.S.A. did not permit to analyze the native strains in the cooperative institution. Therefore, the studies were performed using the viruses isolated in USA and Canada and included preparation of highly purified viruses, extraction of RNA, its labelling by means of conjugation with radioactive iodine (^{125}I) and characterization of RNA segments by means of polyacrylamide gel electrophoresis.
3. In April 1982 the Principal Investigator visited the Cooperating Institution for the second time. This time non-infectious RNA preparations of some Israeli influenza virus isolates were brought to the Cooperating Institution for analysis. This was the first experience of the performance of joint experiments in the Cooperating Institution using viral materials from the Grantee Institution. This experience turned out to be successful and since then a wider set of the RNA preparations from the native influenza virus isolates was sent to the Cooperating Institution.

LIST OF THE PAPERS in which the results of the research
have been published

1. Lipkind, M.A., Weisman, Y., Shihmanter, E. and Shoham, D.
(1979)
The first isolation of animal influenza virus
in Israel.
Veterinary Record, 105: 510-511.
2. Lipkind, M.A., Weisman, Y., Shihmanter, E., Shoham, D.,
Yuval, C. and Aronovici, A. (1979)
Identification of avian influenza virus isolated
from an outbreak of respiratory disease in a
turkey farm in Israel.
Veterinary Record, 105: 534-535.
3. Lipkind, M.A., Weisman, Y., Shihmanter, E. and Shoham, D.
(1979)
Identification of avian influenza viruses isolated
from wild mallard ducks in Israel.
Veterinary Record, 105: 558.
4. Lipkind, M.A., Weisman, Y., Shihmanter, E., Shoham, D.
and Aronovici, A. (1979)
The isolation of Yucaipa-like paramyxoviruses
from epizootics of a respiratory disease in
turkey poultry farms in Israel.
Veterinary Record, 105: 577-578.
5. Lipkind, M.A., Weisman, Y., Shihmanter, E., Shoham, D.,
Douglas, A. and Skehel, J.J. (1980)
Characterization of avian influenza viruses
isolated in Israel 1978-1979.
Comparative Immunology, Microbiology and Infectious
Diseases, 3: 185-192.
6. Lipkind, M.A., Malkinson, M., Akov, Y., Shihmanter, E.
and Shoham, D. (1980)
Retroactive identification as influenza virus of a
hemagglutinating agent isolated in Israel in 1971.
Veterinary Record, 107: 401.

7. Lipkind, M.A., Weisman, Y., Shihmanter, E., Shoham, D., Yuval, C. and Aronovici, A. (1980)
Evidence for interspecies transfer of an avian influenza virus (Analysis of the data on isolation and identification of avian influenza viruses in Israel).
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8. Lipkind, M.A., Weisman, Y., Shihmanter, E., Aronovici, A. and Shoham, D. (1980)
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9. Lipkind, M.A., Weisman, Y., Shihmanter, E. and Shoham, D. (1981)
Isolation of Yucaipa-like viruses from cattle egrets (*Ardeola ibis*) in Israel.
Zentralblatt für Veterinärmedizin, Reihe B, 28: 582-584.
10. Lipkind, M.A., Shoham, D. and Shihmanter, E. (1981)
Isolation of influenza viruses from rock partridges in Israel.
Veterinary Record, 109: 540
11. Lipkind, M.A., Weisman, Y., Shihmanter, E. and Shoham, D. (1982)
Isolation of Yucaipa-like avian paramyxovirus from a wild mallard duck (*Anas platyrhynchos*) wintering in Israel.
Veterinary Record, 110: 15-16.
12. Lipkind, M.A., Weisman, Y., Shihmanter, E. and Shoham, D. (1982)
Review of the three-year studies on the ecology of avian influenza viruses in Israel.
Proceedings of the 1st International Symposium on Avian Influenza; Beltsville, Maryland, USA, April 22-24, 1981, p.p. 69-78.
13. Lipkind, M.A., Weisman, Y., Shihmanter, E. and Aronovici, A. (1982)
Isolation of Yucaipa-like avian paramyxoviruses from migrating coots (*Fulica atra*) wintering in Israel.
Zentralblatt für Bakteriologie, Mikrobiologie und Hygiene, I. Abt. Orig. A253: 159-163.

14. Lipkind, M.A., Shihmanter, E., Weisman, Y., Aronovici, A. and Shoham, D. (1982)
Characterization of Yucaipa-like avian paramyxoviruses isolated in Israel from domesticated and wild birds.
Annalès de Virologie (Inst. Pasteur) 133 E: 157-161.
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Further characterization of H7N7 avian influenza virus isolated from migrating starlings wintering in Israel.
Zentralblatt für Veterinärmedizin (In press).
16. Lipkind, M.A., Hornstein, K., Shihmanter, E., Davidov, K. and Shoham, D. (1982)
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Comparative Immunology, Microbiology and Infectious Diseases (In press).

Papers in preparation

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Characterization of Newcastle disease virus
isolated from migrating starlings (*Sturnus
vulgaris*) wintering in Israel.
2. Alexander, D.J., Lipkind, M., Collins, M. and Shihmanter, E.
Antigenicity and structural polypeptides of avian
paramyxoviruses isolated in Israel in 1979-1981.
3. Lipkind, M., Hornstein, K., Shihmanter, E. and Davidov, K.
Further studies on retrospective identification of
influenza viruses isolated from turkeys in Israel
in the past.
4. Lipkind, M., Shoham, D. and Shihmanter, E.
Isolation of a paramyxovirus strain isolated
from pigs in Israel.
5. Lipkind, M., Weisman, Y. and Shihmanter, E.
Isolation of H1N2 influenza viruses from an
outbreak of a respiratory disease in turkey
farms in Israel.
6. Lipkind, M., Weisman, Y., Shihmanter, E. and Shoham, D.
Isolation of influenza viruses from migrating
coots wintering in Israel.
7. Lipkind, M., Malkinson, M. and Shihmanter, E.
The first isolation of influenza viruses from
domestic ducks in Israel.