Studies* on Avian Viral Infections in Iran

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Summary

Continuos surveillance, since 1974, on avian viral infections using virus isolation procedures, serological techniques and negative contrast electron microscopic examination of pathological specimens, allantoic fluid and cell culture materials as well as gross and histopathological investigations has revealed the presence of a wide variety of viral agents in poultry flocks in Iran. The detected agents are recorded in this communication. Many viruses were isolated, serially propagated and representatives of each group were subjected to identification and characterisation procedures. The presence of some agents has been detected on the basis of other evidence, such as serological results, which is indicated in the text.

Introduction

A knowledge of the avian viral agents present in any country is important since it helps setting limits to diagnoses and, also, is an obligatory requirement for the formulation of regulations on imports and exports. Therefore, we consider it useful to list the avian viral infections which have been detected in this country. Although certain parts of these surveys have been subjects of previous communications, many other agents have been detected but not recorded.

Laboratory works in these studies involved virus isolation procedures, serological techniques, negative contrast electron microscopic examinations

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**Parts of these studies were carried out by the senior author at the School of Veterinary Medicine, University of Shiraz,Iran*

of pathological specimens, allantoic fluid and cell culture materials, also gross and histopathological investigations.

Materials and methods

Embryonated chicken eggs: Specific Pathogen Free (SPF) eggs (Valo, Lohmann, Cuxhaven, Germany) were used for the major part of the work. Eggs from apparently healthy commercial layers (College of Agriculture, University of Shiraz) were used on occasions related to isolation of infectious bronchitis virus (IBV). Embryonated eggs were used where appropriate, and inoculated by standard techniques.
Cell cultures: Chicken kidney (CK) cell cultures were prepared by trypsinizing the kidneys of 3- to 4-week old chicks as described previously(1). Chick embryo liver (CEL) and chick embryo fibroblast (CEF) cell cultures were respectively prepared from 16- and 10-day old embryos, according to the methods outlined elsewhere(2, 3). CK cells were primarily used for isolation, identification and serotyping of conventional avian adenoviruses (Group I). The latter studies were performed using the plaque reduction virus neutralisation technique. CEL cells were employed for the purpose of propagating the egg drop syndrome 76 (EDS 76) adenovirus (Group III), and to prepare antigen for serological tests. IBV and reoviruses were initially isolated in, and adapted to, embryonated eggs. The IBV and reovirus isolates were subsequently adapted respectively to CK and CEF cells, whereby characterisation and serotyping tests were carried out.

Specimens which showed no cytopathic effect (CPE) after the first passage in cell culture were given at least one blind passage of 7-8 days before being considered negative for a virus.
Viruses: The reference virus strains were generously supplied by the Poultry Department, Central Veterinary Laboratory (CVL), Weybridge, Surrey, U.K.
Antisera: Monospecific antisera were obtained from CVL, Weybridge, Surrey, UK, and SPAFAS (Norwhich, Connecticut, USA), or prepared in rabbits and SPF chickens as described elsewhere(2, 3).
Electron microscopy (EM): The procedure followed was that outlined by McFerran et al.(4). In addition to direct examination of allantoamniotic fluid (AAF) suspected of containing IBV, AAF was also harvested about

48 h after inoculation, usually before death of embryos, and centrifuged at low speed. The clarified fluid was layered in 15 ml volumes onto 8 ml of 22% potassium tartrate, followed by centrifugation in an ultracentrifuge with a 25 ml swing out head, at 70,000 x *g* for 1 h. The pellet was resuspended in 1 ml of veronal-buffered saline pH 7.2 and examined, using a Philips 300 EM, within 2 h of preparation.
Type A avian influenza specific cross-reactive antigens: These were prepared according to the techniques of partition and separation of macromolecules in a two-phase polymer system, as described by Albertsson(5, 6).
Investigation of the causal agents: Many viruses were isolated, serially propagated, and representatives of each group were subjected to identification and characterisation procedures. In other cases the presence of infection was detected by serological techniques, histopathological studies or direct electron microscopy of the pathological specimens. In additoin, where applicable, the latter technique was employed for the initial identification of the virus isolates in AAF or cell culture. In most cases, serotyping was performed by virus neutralisation (VN) techniques in cell culture, using either tubes or plastic Petri dishes (plaque reduction test) and also standard antisera.

Results

Coronaviruses: Several strains of IBV, initially isolated in and adapted to embryonated eggs, were subsequently adapted to CK cells(Fig. 1). All the strains fell into the Massachusetts serotype according to virus neutralisation (VN) tests performed in this cell culture system. A serological survey employing a simplified VN test with the Beaudette strain of IBV as antigen, showed that infection with this type of the virus is widespread in chickens.

In addition to Massachusetts type virus strains, there is now evidence indicating the presence of more recently described types or variants i.e. Dutch strains.
Paramyxoviruses: Velogenic strains of Newcastle disease virus (NDV) are endemic in the country.

As yet, there have been no adequate investigations concerning the possible occurrence of other pathotypes of the virus (excluding B^1 and La Sota vaccine strains) and also other paramyxovirus serotypes.

Influenza viruses (*Orthomyxoviruses*): Considering the antigenic variation among type A avian influenza viruses and the fact that it is rarely practicable to include a relatively large number of the recognized subtypes in a serological survey, a panel of 3 subtypes was selected for the preparation of the antigens employed in haemagglutination - inhibition (HI) tests. In addition, partially purified, concentrated, type specific antigens (see materials and methods) were used in agar gel precipitation (AGP) tests.

A considerable number of the sera showed low to moderate titres in HI tests employing the aforementioned subtypes as antigens. However, these sera proved to be negative for specific antibodies following treatment with receptor destroying enzyme (RDE). Also, no positive reaction was observed in AGP tests.

Adenoviruses: Infection with conventional avian adenoviruses (Group I) is widespread and several strains recovered from chickens could be classified into three distinct serotypes(Fig. 2). Outbreaks of egg drop syndrome 76 (EDS 76), caused by Group III haemagglutinating adenoviruses, have been diagnosed in breeders and commercial layers, and reported(2).

Reoviruses: Infection has a wide distribution and 4 strains, belonging to American strain S 1133 serotype, have been isolated from cases of arthritis/tenosynovitis and malabsorption syndrome(Fig. 3) in chickens, and characterised(3).

Herpesviruses: Marek's disease virus (MDV) infection is widespread,judged by clinical and histopathological findings.

Outbreaks of infectious laryngotracheitis (ILT) are relatively common, particularly in commercial layers and breeders.

Birnaviruses: So called standard strains of infectious bursal disease virus (IBDV) were first isolated in the early 80s. More recently, highly virulent strains of the virus capable of causing up to 90% mortality in 5- to 6-week old SPF chickens have emerged and are currently under study.

The infection is widespread and, therefore, live as well as inactivated oil emulsion vaccines are now employed.

Picornaviruses: Infection with the avian encephalomyelitis virus is widely distributed. Live vaccines are used in breeders.

The presence of avian nephritis virus (ANV) is suspected in very young chicks on the grounds of pathological findings.

Poxviruses: Infection of domestic fowls, turkeys, pigeons and canaries with

poxviruses have been diagnosed by virus isolation and, also, direct negative contrast electron microscopy.

Retroviruses: The presence of RNA tumor viruses has been recorded based on clinical and histopathological evidence.

Pneumoviruses: The presence of the infection is highly suspected on the basis of results from serological test carried out on breeders.

Chicken anaemia virus (CAV): The condition have been strongly suspected in commercial replacement chicks, on the basis of clinical, haematological, gross pathological and histopathological findings.

Discussion

The main targets of avian virology might be defined as: to establish which viral agents are present in any country, to evaluate the pathological potential and economic significance of the existing viruses and, ultimately, to eradicate these agents or, at least, by appropriate means minimise the economical losses they cause. Continuous surveillance of the viral infections of avian species with particular emphasis on poultry is important in any country because the information may serve as a guide to determine limits to diagnosis; it is required for setting up regulations concerning import and export and, in addition, it is most desirable to establish unequivocally the presence of a virus before a sound and logical vaccination strategy can be formulated.

The above goals are not easily attainable for various reasons. First of all, it would be illogical to assume that all economically important avian viruses have been discovered. Secondly, some of the known viruses can be isolated only with great difficulties. In this regard, even though the systems employed were adequate to propagate viral agents, problems arose in detecting their presence. Thus, some IBV strains failed to cause dwarfing of chick embryos in initial passages whereas in AAF examined by negative contrast electron microscopy, the virus could be detected. However, routine examination of AAF using the electron microscopy is time consuming. In the same context, to detect adenoviruses efficiently, at least one blind passage was necessary in cell culture. Chicken anaemia virus is fastidious and as many as 6 blind passages of MDCC-MSB[1] lymphoblastoid cell line materials are sometimes required for primary isolation of the virus.

Many a virus is isolated under certain circumstances which leads us to

33000X3.7

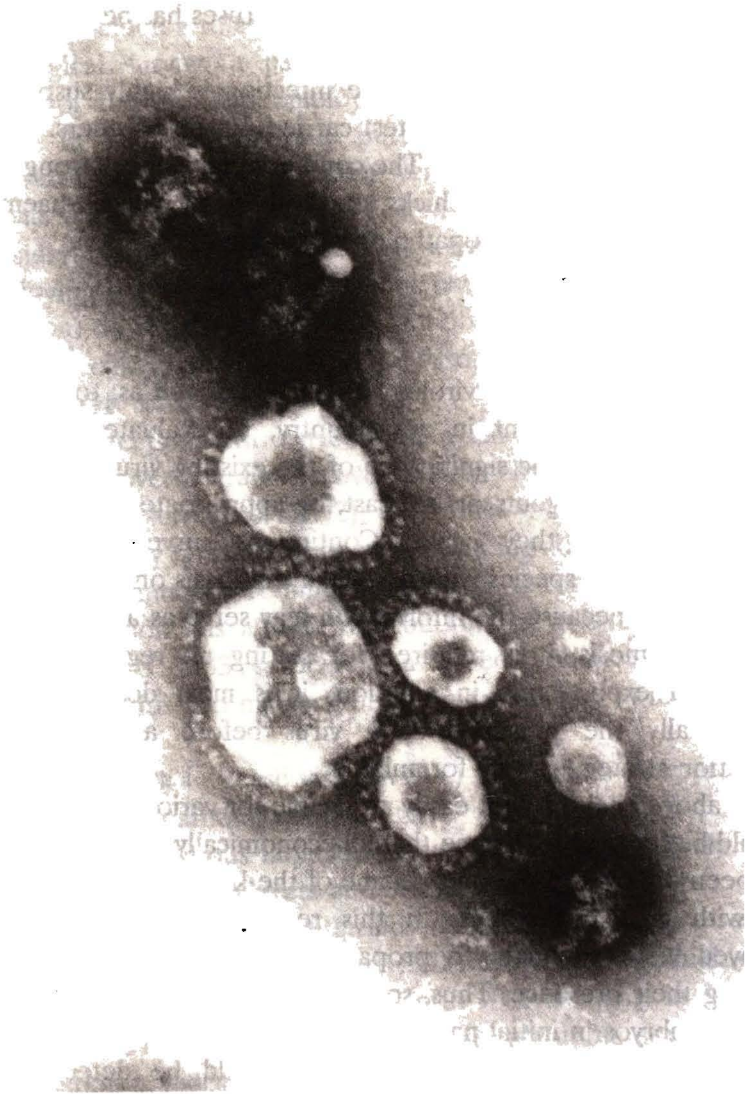
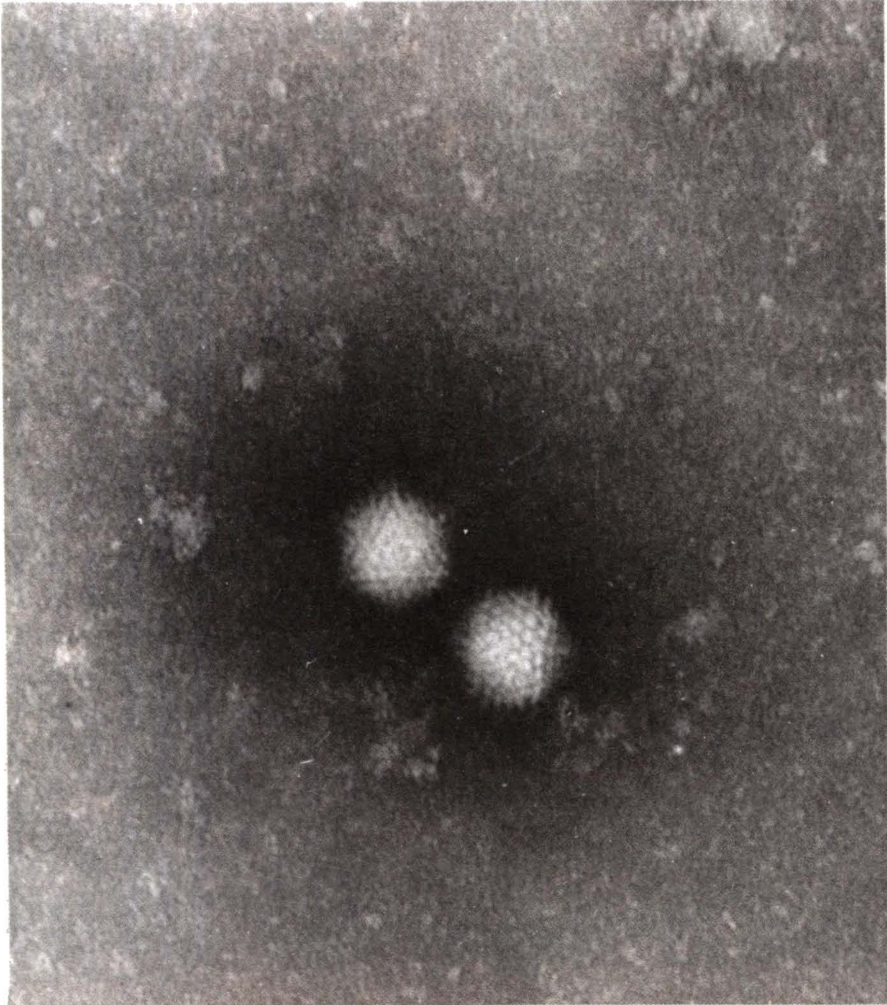


Fig 1. Infectious bronchitis virus. x 120,000

70000X2-8



ig 2. Adenovirus. x 196,000

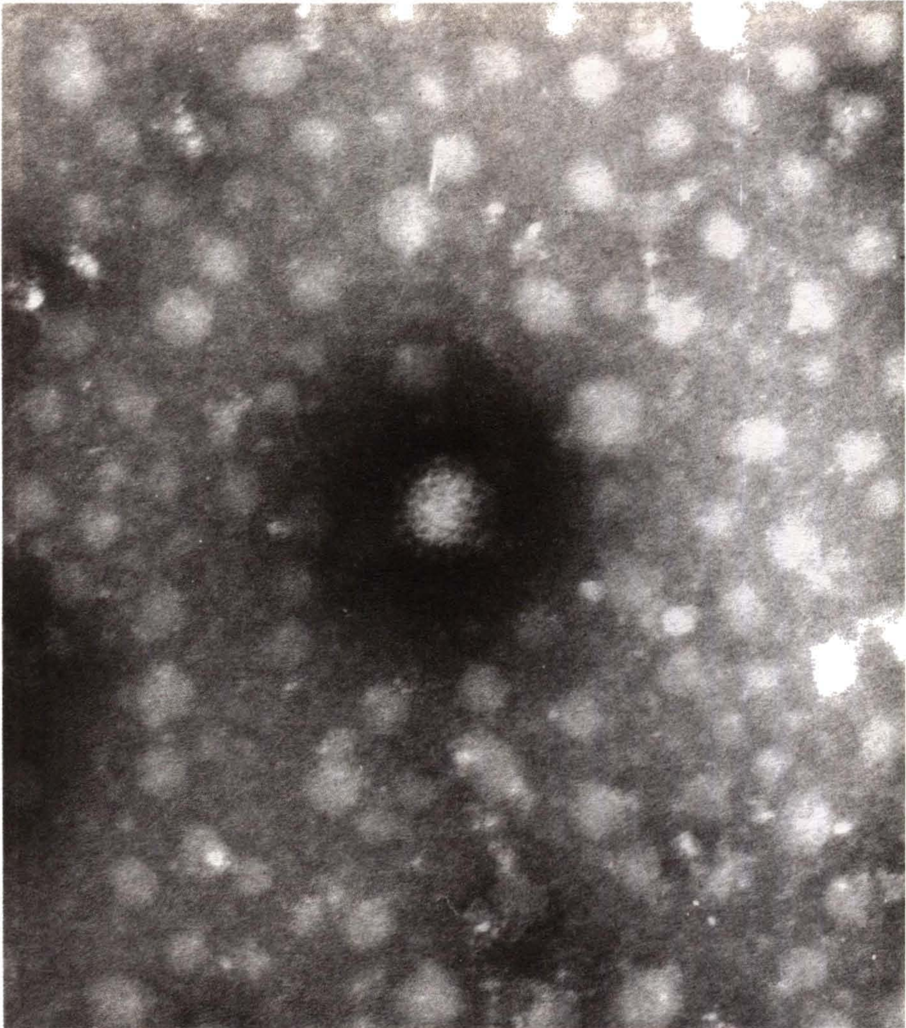


Fig 3. Reovirus. x 190,000

believe that it is the causal agent of the disease under investigation, but subsequently fails to reproduce the respective symptoms and lesions in experimental infections probably due to difficulties, under laboratory conditions, to create appropriate environmental factors.

It is likely that most avian viral agents detected can survive indefinitely and it is reasonable to assume that once a viral infection is established in the poultry population within a country the agent can be regarded as indigenous unless proved otherwise.

Although our studies of the avian viral infections cover a period of about tewnty years, and include the collection and examination of a large number of specimens, we do not imply that the techniques employed or samples taken have been ideally comprehensive to detect all avian viral infections present in this country.

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