

RESEARCH COMMUNICATION

ISOLATION AND IDENTIFICATION OF ADENOVIRUS 127, THE CAUSATIVE AGENT OF EGG DROP SYNDROME (EDS), FROM COMMERCIAL LAYING HENS IN SOUTH AFRICA

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ABSTRACT

BRAGG, R. R., ALLWRIGHT, D. M. & COETZEE, L., 1991. Isolation and identification of Adenovirus 127, the causative agent of egg drop syndrome (EDS), from commercial laying hens in South Africa. *Onderstepoort Journal of Veterinary Research*, 58, 309-310 (1991).

A drop in egg production associated with loss of pigment and soft-shelled eggs was experienced on a farm in the Western Cape. Investigations revealed high levels of antibodies against adenovirus 127, the causative agent of egg drop syndrome (EDS) in chickens which were not vaccinated against EDS. A cytopathic agent was subsequently isolated in chicken embryo liver cell cultures and identified as EDS virus by haemagglutination inhibition and neutralization tests.

A disease condition characterized by soft shelled or shell-less eggs was first described in the Netherlands in 1973 (Van Eck, Davelaar, Van den Heuvel-Plesman, Van Kol, Kouwenhoven & Guldie, 1976). Viruses which were isolated in Northern Ireland in 1978 (McFerran, McCracken, McKillop, McNulty & Collins, 1978) were shown to produce a disease similar to that described by Van Eck *et al.* (1976).

Although EDS is widespread in waterfowl and domestic ducks and geese around the world (McFerran, 1989), EDS virus has never been isolated in South Africa.

In early December 1990, a farmer in the Paarl district of the Western Cape recorded a dramatic drop in egg production in a house with about 10 000 birds in a battery system. The eggs were depigmented and soft-shelled or shell-less, many having a sandpaper-like texture. By the end of December the four adjacent houses on the site were all affected. Besides the changes in egg production and shell quality, no other signs of disease or mortalities were recorded. The farmer thus suspected nutritional problems and added extra calcium to the feed. As there was no improvement, the farmer requested assistance.

The birds had been purchased as day-old chicks from various suppliers and were reared to point-of-lay on a nearby rearing farm. They were vaccinated against the following diseases: Marek's disease, Newcastle disease, infectious bronchitis, laryngotracheitis, fowlpox, infectious coryza, infectious bursal disease (Gumboro), and *Mycoplasma gallisepticum*. The birds were reared in wire cages for most of the rearing period and were moved to batteries at about 18 weeks of age. Different breeds of birds were kept in each of the four houses and they were of different ages. Feed was supplied manually in troughs and water by an automatic cup system.

Blood samples were collected at random from birds in each of the 4 houses and the serum was collected. The birds were known to be positive for both *M. gallisepticum* and *M. synoviae*. A commercial ELISA test kit (Whittaker Bioproducts) was used to test the serum for antibodies to Newcastle

disease and infectious bronchitis. The antibody results obtained for these diseases were within the normal limits. The sera were tested for antibodies against EDS virus by the haemagglutination inhibition (HI) test according to the methods described by McFerran (1989) with modifications. The serum was not diluted 1/10 with PBS but was used undiluted. A reaction time of 30 min, instead of the 15 min quoted by McFerran (1989) was allowed and a 0.3 % suspension of chicken erythrocytes was used. [Reagents supplied by the Poultry Reference Laboratory (PRL)]. Titres in the range of log₂ 7-14 were obtained. Serum samples were submitted to the PRL for confirmation where identical results were obtained.

From the clinical signs on the farm as well as the serological evidence of the presence of EDS virus, a diagnosis of egg drop syndrome, caused by EDS virus was made. Samples of blood which had been collected in heparin were submitted to the PRL for virus isolation as a final confirmation.

The blood samples were centrifuged in a bench top centrifuge and the buffy-coat cells were carefully collected and stored at -20 °C until they could be inoculated onto cells.

EDS virus replicates most readily in cells of duck or goose origin, but it also grows well in chicken embryo liver cells (Smyth & McFerran, 1989). Due to problems in obtaining suitable duck or goose material, attempts were made to isolate the virus in chicken embryo liver (CEL) cells grown from SPF eggs (supplied by Immunovet Services). The CEL cells were produced according to standard techniques and were grown in 25 cm² tissue culture flasks. The medium used consisted of M199 supplemented with 10 % fetal calf serum (FCS). The medium in the flasks was replaced the day after the cells had been produced with the same medium used to grow the cells. Once the cells were confluent, the virus samples were thawed, diluted 1/10 with Eagles minimum essential medium (MEM) and filtered through a 0.45 µm membrane filter. The medium was removed from the cultures and the virus suspensions were inoculated onto the cells. The cells were incubated at room temperature for 30 min to allow adsorption of the virus to the cells. Thereafter, the excess inoculum was discarded and the cells were fed with fresh medium containing 2 % FCS. The cells were incubated at 37 °C for 7 days and were examined daily for the presence of cytopathic effects (CPE).

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The first evidence of CPE was detected three days after inoculation. Two days later, all the inoculated cell cultures showed advanced CPE. A sample of the fluid was harvested from each flask and a haemagglutination test was carried out. Positive haemagglutination was found in all of the undiluted samples and in 3/6 of a 1/2 dilution of the samples. No agglutination was found at higher dilutions. It was decided that the HA titre was not high enough to perform a haemagglutination inhibition (HI) test. The samples were frozen and thawed three times and were re-inoculated onto fresh CEL cells. Following sub-culture, samples showing advanced CPE had HA titres in the region of \log_2 5–6.

An HI test was carried out according to standard procedures using positive and negative serum against EDS virus. Positive serum was either produced in the PRL, or was imported from the USA. It was found that the positive serum inhibited the agglutination of the chicken erythrocytes, thus confirming the identity of the isolate as EDS virus (Adenovirus 127).

A neutralization test was also carried out according to standard techniques and it was found that when the isolated virus was mixed with EDS positive serum, no CPE were produced, while the samples mixed with EDS negative serum did cause CPE.

It can thus be concluded that a virus similar to EDS virus was isolated from a flock of birds which were showing classical signs of EDS and which also

showed high levels of antibodies specific for EDS virus. The virus which was isolated from these birds was identified as EDS virus by virtue of the results obtained by the HI test as well as the neutralization test.

ACKNOWLEDGMENTS

The authors would like to thank Mrs M. E. Combrink for the production of the CEL cell cultures as well as Mrs L. Coetzee and Miss Van Aswegen for their technical assistance with the HA and HI tests.

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