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RECENT OUTBREAKS OF FOWLPOX IN THE SUDAN: ISOLATION OF THE VIRUS AND EMPLOYMENT OF THE MICRO GEL IMMUNODIFFUSION TEST FOR ITS SERODIAGNOSIS

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INTRODUCTION

Fowlpox (FP) is an infectious disease of the fowl characterized by wart-like nodules on the unfeathered parts of the body, or it may manifest itse'f in a diphtheric form, which is characterized by the formation of pseudomembranes in the mouth and throat of the affected birds. However, the presence of both forms of the disease is not uncommon.

The disease is caused by a virus which belongs to the genus Avipoxvirus of the family Poxviridae.

The first record of the disease in the Sudan was made in 1936 (Anon). Since then the disease has regularly been reported in the country. In spite of this, scanty research has been conducted in this field, (Khogali 1972; Elamin et al 1980).

A live vaccine incorporating the Beaudette strain (see materials and methods below) is produced locally in the Sudan.

Diagnosis of the disease in the Sudan, has largely depended on the clinical picture and growth of the virus on the chorioallantoic membrane (CAM) of embryonating chicken eggs, rather than on a rapid serological test.

The purpose of the present work was to develop a rapid and simple serological test for the routine diagnosis of the Fowlpox virus in the Sudan.

MATRIALS AND METHODS

The reference antigen and Antiserum:

The reference fowlpox antiserum was kindly supplied by Dr. Alexander of the Central Veterinary Laboratories, Weybridge, U.K.

The Beaudette vaccine FP virus strain was used as the reference antigen in this study. It is a chick embryo attenuated virus

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which is used as vaccine in the Sudan. It was procured from Kabete laboratories, kenya. In 1970 (Prof. B.E. Alipersonal communication).

Clinical Signs:-

The head and the buccal cavity were the only parts involved in sick birds. Wart like lesions were seen on the comb and watles. Diphtheric membranes with excessive mucoid salivation were seen in the buccal cavity. Conjuctivities and swelling of the eyelids were characteristic features. In certain cases the eyelids were closed and excessive lachrimation was seen. Birds were listless, anorexic, sneezing frequently and shedding plenty of mucus and saliva in the premises. Some birds had difficulty in breathing and were gasping due to the presence of thick mucus in their nostrils.

Using sterile surgical instruments, saliva and mucus from the buccal cavities of sick birds were collected. Pock lesions were also removed and all wre placed in sterile vials containing glycerol buffered saline (GBS) containing penicillin (1000 1U/ml), streptomycin (1000 Ug/ml) and Mycostatin (50U/ml). Samples were ground aseptically and centrifuged at 1000 rpm for 15 min. The supernates were collected and stored in GBS at - 70 C The supernatant fluids were incculated on the CAM of 11 days old chick embryos as described earlier (Abu Elzien 1976). Further two passages were performed on the CAM and the virus titres wre calculated as pock forming units (PFU/ml) as previously described (Abu Elzein 1976). This virus was designated (FPZ/84).

Preparation of the anti FPZ/84 virus antiserum:

One ml of 108 PFU/ml virus was homogenized with an equal valume of complete Freunds, adjuvant and inoculated intramuscularly into a sheep (0.5 ml on each thigh). Two weeks later serum was collected and a similar booster dose adminstered. After 2 further weeks serum was again collected.

Virus identification using the gel immunodiffusion test (GID):

The slide method of the GID was carried out using 1% Noole agar (Difco Laboratories) in phosphate bufferedsaline (PBS), pH 7.4. The test pattern consisted of a six well rosette round a central well. Each well was 3mm in diameter with a distance of 5mm between the middle of the central well and middle of peripheral wells. Using capillary tubes the reference anti FP antiserum was added to the central well Test samples, suspected to contain FP virus, were added to alternate wells. To the wells between these the FP Beaudette strain was added. Non-immuned chidken sera and non-inoculated CAM suspensions were used as controls. Slides were then placed in a humid chamber and examined next day for the presence of precipitin lines.

Test of the specificity of the sheep anti FPZ/84 hyperimmuned serum:

To check on the specificity of the sheep hyperimmuned serum, the serum neutralization test was was carried out in 11 day old chick embryos as described by Mascoli & Burrel (1965). The hyperimuned serum was reacted separetly against its homologous virus and also against the reference strain. Non-immuned sheep serum was used as a control.

Detection of FP antibodies in sera from randomly-sampled chickens using the GID test:

To examine the validity of the GID test for the detection of antibodies against the FP virus, sera from apparently healthy chickens were reacted against the FP virus in the GID test such that the virus antigen was placed in the central well. The test sera and the sheep hyperimmuned serum were placed alternatively in the peripheral wells. Non-inoculated CAM suspensions and non-immuned sheep serum were used as controls.

RESULTS

History of the outbreaks:

In February 1984, severe outbreaks of suspected FP involved laying hens in 5 private farms at Omdurman district, Khartoum Province. the mortality rate was 20%, while the morbidity rate was 80%. No history of vaccination against FP was available in the records of the affected farms.

Virus identification:

A discernible precipitin line was produced between the test virus and the reference FP antiserum. The line merged with the line produced betwwen the reference virus and the reference FP antiserum forming a line of complete identity (Fig. 1). No lines were seen in the negative controls.

Table I shows the results of the samples from the outbreaks at Omdurman.

Specificity of the sheep anti FPZ/84 Hyperimmuned serum:

The sheep hyperimmuned serum has completely neutralized its homologous virus and the reference virus strain. Non-immuned sheep serum could not neutralize infectivity of the two viruses.

Detection of the FP antibodies in chickens sera:

As shown in table 1, 50% of the sera had antibodies against the FP virus.

DISCUSSION

Clinically, FP is known to exist in the Sudan since 1963 (Anon), and yet no routine serological test was available to give a rapid identification of the causeal virus.

The present study has proved that the micro GID is rapid, sensitive, economical, easy to perform and is able to give an answer within 18 hours on samples from naturally-infected birds, and could thus be used routinely for the serodiagnosis of the FP virus in the Sudan.

The FPZ/84 field virus isolate was serologically indistinguishable from the vaccine strain. This could be seen in the complete lines of

identity produced between the reference antiserum and each of the tested viruses, confirming that the field isolate was a FP virus.

As several variable factors may interfere in the GID tests the specificity of the hyperimmune serum used in such test should be well studied. Realizing this, the sheep hyperimmune serum used in our experiments was subjected to rigor specificity tests, and was found specific for the FP virus. This has been reflected by its ability to completely neutralize the infectivity of both its homologous virus and that of the reference strain. Non-immune sheep serum was unable to neutralize the infectivity of the virus.

The validity of the micro GID test, for the detection of antibodies against the FP virus, was checked by its ability to detect such antibodies in the sera which were randomly collected from apparently healthy chickens.

In conclusion, specific hyperimmune sera against the FP virus could be despached to regional laboratories, in the Sudan where diagnosticians may perform the simple GID test for the diagnosis of the suspected cases of FP locally. Indeed, this will save time and energy and is expected to exclude the remote possibility of spreading infection by sending samples to the central laboratories at Soba, Khartuom.

SUMMARY

The micro GID test was successfully employed to diagnose the FP virus in the Sudan. Results could be obtained within 18 hours.

Antiserum against the isolated virus was produced in sheep in lage quantities, and its specificity was assessed using the serum neutralization tests.

All samples collected from the naturally-infected birds were positive for the FP virus in the GID test.

It is suggested that specific sera against the FP virus could be despached to regional laboratories where the micro GID test may be used for the serodiagnosis of the FP virus infection.

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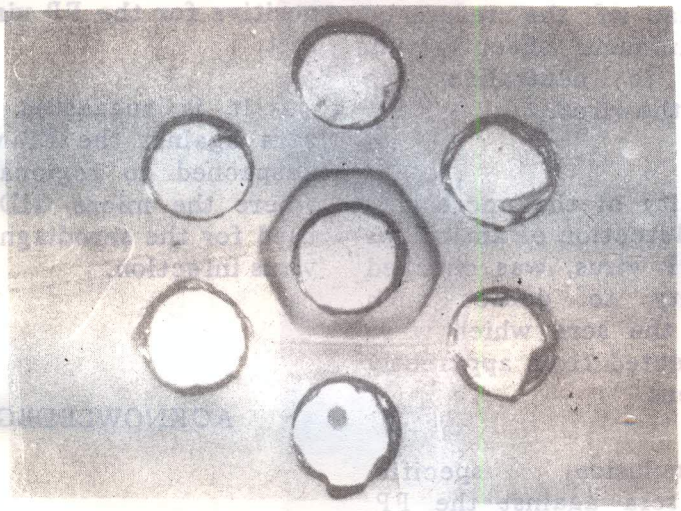

Fig. I
Comparing the reference FP virus with the FPZ/84 field virus isolate using the GID test.
Central well = Reference FP ant-iserum.
Wells 1,3,5 = Reference Fp virus.
Wells 2,4,6 = The FPZ/84 field virus isolate.

Table 1. Detection of the precipitating antibodies in the sera of the randomly collected chickens; and the AGID results on the samples from birds from the FP outbreaks.

Sample	No. Tested	% with precipitating anti fowlpox antibedies
Sera	26	50%
		% with FP virus precipitating antigens
Pox lesions & exudates from sick birds.	15	100%