The Sudan J. Vet. Res. (2004), **19***:* 23-27
With two figures in the text

Typing of Some Field isolates of Infectious Bursal Disease Virus by the Agar-gel Precipitation Test

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ملخـــص البحث

تم تصنيف ست معزولات حقلية من فيروس مرض جراب فابريشص المعدي (bursal infectious) diseases ومقارنتها بعترة كلاسيكية معتدلة الضراوة بواسطة إختبار الترسيب في هلام ألأجار (AGPT) ومضاهاة خطوط الترسيب المتكونة بين المستضد والضد للمعزولات المختبرة0 أثبتت الدراسة أن أغلب الفيروسات الحقلية أشد ضراوة من العترة الكلاسيكية وتنتمي الي نوعها المصلي رقم 1

Summary

Antigenic and pathogenic classification of six IBDV field isolates by the shape and size of the precipitin lines formed between them and a classical mild strain in the agar-gel precipitation test showed that the fields viruses belonged to serotype-1 classical virus. In addition, most of the field isolates examined were more virulent than the classical IBDV strain.

Introduction

Infectious bursal disease (IBD) is a highly contagious disease of young chickens with worldwide distribution. The bursa of Fabricius is the main target organ where severe inflammatory changes take place. Based on virus neutralization test, the IBD virus strains have been grouped into two serotypes; 1 and 2 (Jackwood *et al.,* 1988).

In USA Snyder *et al.* (1988) confirmed the emergence of variant strains which were not neutralized by classical monoclonal antibodies suggesting an antigenic shift among the viruses. In the Sudan, the IBD was firstly observed in late December 1980 in six–week-old chickens at El Obied town. Mortality rate was 3% in six-week-old chickens and 22% in one-day-old chicks (Shuaib *et al.*, 1982).

The main purpose of this study was to classify some Sudanese IBDV field isolates antigenically, depending on the type and size of their precipitin lines in the Agar-gel Precipitation Test (AG P T).

Materials and Methods

IBD viruses:

i- Field isolates:

Six IBD viruses isolated from field outbreaks were collected during 1994-1997. They were isolated from Butri, Gezira and Arab Poultry Company. A 20% bursal suspension in phosphate buffered saline (PBS) prepared from each specimen, each representing a viral field isolate, was used as an AGPT antigen.

ii- Standard antigen:

The standard antigen was kindly provided by Dr. Amal Mustafa, Head Department of Avian Pathology, CVRL, Soba, Khartoum. It was a lyophilized AGPT antigen provided in 1 ml aliquots. Before use the antigen was reconstituted in 1 ml deionized distilled water, it was further diluted to1:2 in PBS and used in the test as the positive control classical IBDV.

iii- Antiserum:

A strong AGPT- positive serum collected from a rabbit inoculated with an IBD vaccine was used as the positive IBD virus antiserum.

Test Procedure:

An offset linear pattern of nine inner and eight outer wells with six mm diameter and 3mm interspaces, was used. Alternatively, wells in the outer two vertical rows were filled with the six test antigen (T_1 – T_6); the remaining outer wells were filled with the positive known standard antigen. The central wells were filled with the antiserum. The plates were incubated at room temperature in a humidified chamber for 48 hours. They were then examined in front of an illuminating source of light for presence of precipitin lines.

Holes were also cut in the shape of a circle containing six-outer and one inner well. The Field and control IBDVs antigens were placed in the outer wells in an alternative pattern while the inner well contained the antiserum.

Results

Results of AGPT are shown in Figs. 1 and 2. Close examination of the precipitin lines revealed that they were continuous and had curved points of contact. No angulations or spurs were observed at the points of contact between wells. Moreover, the precipitin lines that had developed from field isolates were similar in shape and larger in size than those formed by the classical mild strain which was used as a control.

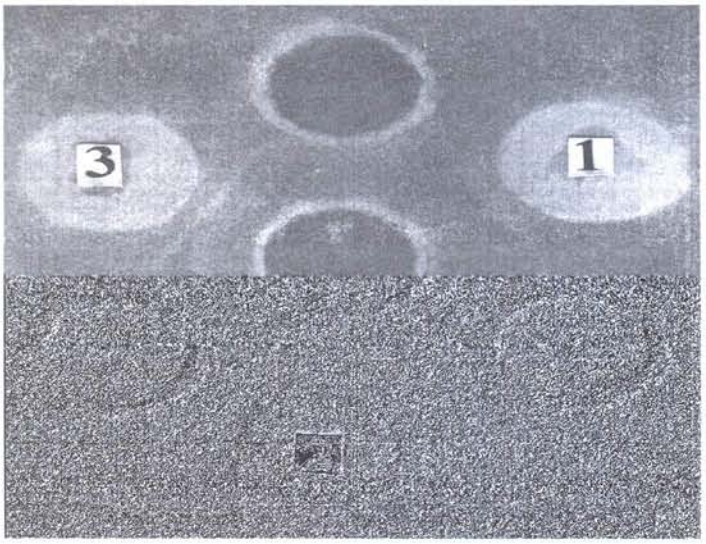

Fig.1: AGPT precipitin lines formed by IBDV standard (Classical) strain and three field isolates (1, 2 and 3)

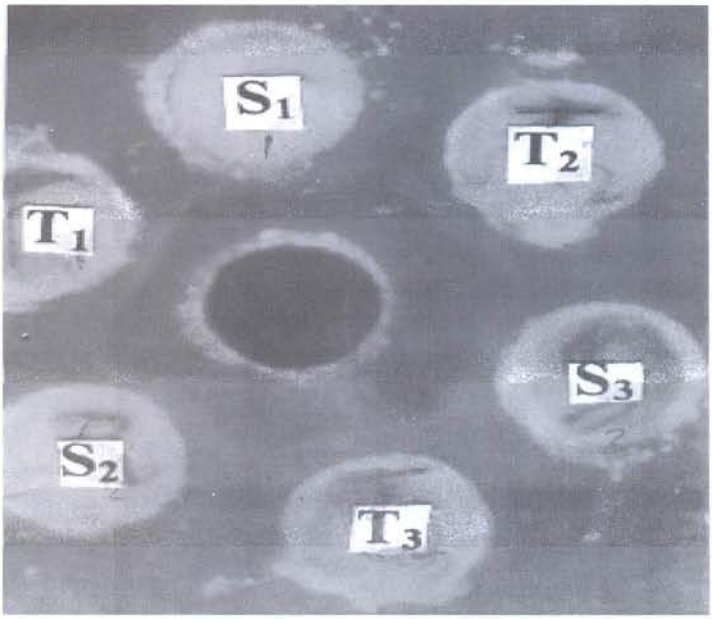

Fig. 2: AGPT precipitin lines formed by IBDV standard (S_1, S_2 and S_3) and three field strains (T_1, T_2 and T_3).

In Fig. 1, the black wells represent the standard IBDV strain while the white ones represent the three IBDV field isolates of Butri, Gezira and Arab Company in clockwise direction starting from the upper well. Fig. 2 shows the precipitin lines which were formed by the standard IBDV strain and three other test field isolates of IBDV. .

Discussion

Six IBD field viruses were antigenically and pathologically classified by the AGPT. Results presented in figs. 1 and 2 show that the precipitin lines produced by the six virus isolates fused with each other and with the classical IBDV strain. Moreover, no spurs or angulations were observed on the precipitin lines.

According to Wyeth and Chettle (1988), all IBDV strains share a common gel diffusion antigen irrespective of virulence; standard strains form continuous lines at the points of contact in the AGPT and variant strains form continuous lines too but points of contact between wells are angulated with small spurs. The result of this investigation was accordingly assessed, i.e. all the Sudanese IBDV field isolates tested belonged to the serotype 1 of the classical strain.

Our results also agree with those of Takase *et al.* (1993) who, in a relevant investigation using 29 IBDV strains, could pathologically classify them into three groups according to the patterns of their precipitin lines. In this investigation it was noticed that despite the continuation of the lines formed by all IBDV field isolates and classical virus yet the sizes of the lines were different. Precipitin lines formed by the field viruses were larger in size than those formed by the classical strain which was a known mild classical (commercial) virus (Fig. 1 and 2). Moreover, most of the field isolates formed lines slightly differing in size. Hence it may be concluded that the local IBDV field isolates were more virulent than the classical strain.

It has been found that there is a closer relationship between the highly virulent and virulent classical viruses than that between the former viruses and the mild tissue culture adapted ones (Kibenge *et al.*, 1991). Thus Sudan recent local isolates are more related to virulent IBD viruses than to mild ones. However, some investigators had different opinions on this subject. Of these Reddy (1994) who has found that the inability of the polyclonal antisera to discriminate between the serotypes and subtypes in cross-immunoprecipitation, was due to the big structural similarities among viral proteins.

Unfortunately, cross neutralization assays which are considered ideal for a large scale antigenic study on the antigenicity of field isolates, could not be done during the present study since these viruses failed to replicate in tissue culture and monoclonal antibodies were not available for their

classification. Thus the work described here was only an attempt to classify our local isolates under our limited local facilities. More advanced approaches are needed.

Acknowledgements

The authors are grateful to Director, Central Veterinary Research Laboratories and Director General, Animal Research Corporation for permission to publish this work.

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