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# *STUDIES ON RINDERPEST LIKE DISEASES AND THEIR COUSES*

*(With One Table and One Figure)*

*By*

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دراسات عن الفيروسات الشبيهة بفيروس الطاعون البقرى

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تم عزل إثنتان وعشرون عترة من فيروس التهاب الأنف والقصبة الهوائية المعدى من الأبقار وبمقارنة هذه المعزولات مع عترات المرجع وهي : الكلورادوا والبرامون والهنجريان باستخدام الأنزيمات المختلفة لتحليل الحامض النووى ( DNA ) وهي:

Eco RI, Bgl II, Ban HI, and Hind III.

وجد أنها تنسبب الى نوعين هم الكلورادوا (ستة معزولات) والبرامون (ستة عشر) للذلك يعتبر الحامض النووى للفيروس الطريقة المثالية لايجاد التناسب بين مختلف العترات .

## *SUMMARY*

Twenty-two isolates of infectious bovine Rhinotracheitis, virus, were isoalted from genetal and Respiratory tract, and conjunctiva ofcattle.

These isolates were compared with reference strains of IBRV (Colorado, Paramon & Hungerian strains) using restriction endonucleuse enzymes, (Eco $R_1$, $Bg_{I,II}$, Bam HI and Hind III).

Our result revealed that six isoltes were related to the Colorado (type) while the Paramon, (16 isolates reference were detected.

No isoalte was related to the Hungerian strain.

## *INTRODUCTION*

Bovine herpesvirus-1 (BHV-1), the causal agent of infectious bovine rhinotracheitis (IBR) and infectious pustular vulvovaginitis (IPV), is a member of the alpha herpesvirinae subfamily. It is similar to other herpesviruses possesses a linear double stranded DNA genome which has amolecular weight of approximately $10^8$ (FARLEY et al., 1980). Also like other herpesviruses, IBR can remain latent in animals, probably in trigeminal ganglions and can be reactivated with relative ease (HOMAN et al., 1980). IBVR is an important pathogen of Cattle and can cause severe respiratory infections, vulvovaginitis, aborations, conjunctivitis, meningiencephalitis and generalized systemic infections (GIBBS and REWEYEMAMU, 1977).

Infectious bovine rhinotracheitis was first recognized in 1950 (MILLER, 1955) in Colorado foodlot cattle. Thus IBRV represents a good model for studying the biology and immunology of active latent herpesvirus infections in natural hosts.

Many attemps have been made to differentiate strains of infectious bovine rhinotracheitis, especially those of respiratory and genetal origin (IBR & IPV). Most attempts, revealed that different isolates are quite similar with respect to biophysical and antigenic properties. (GILLESPIE et al., 1959; WAGNER and GILLESPIE, 1959, MCKERCHER et al., 1959, LIESS et al., 1960, MIKERCHER, 1963, MCKERCHER, 1964a. MOHANTY & LILLIE, 1970), could not observe any difference between the morphology and the intracellular development of the strains they studied nor did BLACK and SLACK (1972), in comparing the base composition of the deoxy-ribonucleic acids (G+C=72%). Plaque produced under agarose, could distinguish strains isolated from encephalitis (BAGUST, 1972), whereas the other strains could not be differentiated by this technique (MCKERCHER, 1964, BUENING & GRATZEK, 1967 and BAUST, 1972). BARTHA et al. (1969) observed differences in the resistance to heat & to trypsin treatment of certain strains. Slight antigenic variations can be observed using neutralization kinetic studies (GRTZEK et al., 1966, BUENING & GRATZEK, 1967, CRANDELL, 1972, HOUSE, 1972, POTGIETER & MORE, 1974) as well as biophysical differences by zone electrophoresis (STRAUB and BOHM, 1962). To determine whether similar differences occur between the local isolates and the reference strain we compared several strains isolated from genetal and Respiratory & conjunctival tracts of cattle with the international reference strains (Colorado & Hungerian) and local reference strain (paramon).

The present study discusses the differences in cleavage site of DNA of these isolates using restriction endonuclease enzymes.

## *MATERIAL and METHODS*

**Cells :**

Madin-Darby Bovine Kidney (MDBK) cells were grown in minimum essential medium (MEM), supplemented with 10% fetal calf serum.

**Viruses :**

1- Reference viruses: The Cooper of (Colorado-1) strain of BHV-1 was kindley supplied by the veterinary Diagnostic laboratories, Ames, Iowa, U.S.A. The Paramon strain was obtained from Virology Department of Animal Health Research Institute, Dokki, Giza. Hungerian strains was received from Prof. Dr. Baritha A., Hungarian Academy of Science.

2- Isolated viruses: Twenty-two isolates of IBR-virus were isolated in the present work.

**Restriction endonuclease analysis of viral DNA:**

a) DNA extraction: was carried out according to MISRA et al. (1983).

b) Restriction endonuclease analysis (RE), according to OSORIO et al. (1985).

## *RESULTS*

**Restriction endonuclease analysis of viral DNA :**

Figure (1 a) shows the cleavage of DNA of the local BHV-1 isolates as well as the reference strains produced by Eco R1 restriction endonuclease enzyme. This enzyme cleaved DNA of 6 BHV-1 isolaes into 7 fragments and the other 16 isolates into 6 fragments. The same results were obtained in reference strains Colorado and Paramon strains respectively, while the DNA of Hungerian strain was cleaved into 5 fragments. Also the restriction endonuclease enzyme Bgl gave the same results obtained by Eco RI but differed in the migration patterns (Fig. 1 b).

The restriction endonuclease Hind III cleaved the local BHV-1 DNA into 11 fragments (6 isolates) and 10 fragments (16 isolates). Also this enzyme cleaved the DNA of the reference strains Colorado and Paramon into II and 10 fragments respectively. But its cleavage to the DNA of the Hungerian strain lead to 8 fragments (Fig. 1 c). The Bam HI cleaved the DNA of the local isolates into 9 fragments in 6 isolates and 8 fragments in the other 16 isolates. Also this enzyme cleaved the DNA of the reference IBR strains; Colorado, Paramon and Hungerian strains into 9, 8 and 4 fragments respectively (Fig. 1 d). From these obtained results our isolates seemed to be related to two types which are colorado strain (6) and Paramon strains (16). No isolate was related to Hungerian strain. It was found that the analysis of DNA by different restriction endonuclease enzymes supported the results obtained by those of viral protein analysis in classification of the local BHV-1 isolates.

The local isolates, as well as the reference strains, source of isolation, type of restriction enzyme used and number of fragments are shown in Table (1).

From the above mentioned data our isolates seemed to be related to two types of strains which are colorado (6 isolates) and paramon (16 isolates). So, restriction endonuclease enzymes analysis of viral DNA appears to be an appropriate tool to find out the relatendness of different BHV-1 isolates.

## *DISCUSSION*

Bovine herpes Virus-1 (BHV-1), commonly known as infectious bovine rhinotracheitis virus, is a prominant cause of respiratory disease, abortion, conjunctivitis and pustular vulvovaginitis in cattle (KAHRS, 1981).

Restriction enzyme analysis of viral DNA appeared to be an appropriate tool for differentiation of BHV-1 isolates (Table 1).

The restriction enzymes Eco $R_1$ cleaved the DNA of 6 of the isolates (ST 143/88, ST 156/88, ST 177/88, AH 198/88, AH 205/88 and AA 352/88) as well as the Colorado reference strain into 7 fragments and the other 16 isolates (AH 2/88, AH 30/88, AH 31/88, AA 321/88, AA 321/88, AA 32/88, AA 367/88, AB 401/88, AA 406/88, AA 416/88, AA 428/88, AA 434/88, AA 452/88, AH 455/88, AH 467/88, AH 470/88, AH 470/88) as well as the Paramon reference strain into 6 fragments. The DNA of

Hungerian reference strain was cleaved into only five fragments. Our results are in-agreement and extended the findings of MAYFIELD et al. (1983) who found that the purified Cooper strain DNA was cleaved with restriction. Ecor $R_1$ into 7 fragments and designated them as A,B,C,D,E,F & G and the findings of MISRA et al. (1983) who found that the restriction endonuclease Eco $R_1$ cleaved BHV-1 DNA into six or seven fragments, also found in their analysis of 116 BHV-1 isolates, that differences in cleavage patterns were not associated with different disease syndrome. Three cleavage pattern were found among the 116 isoltes, and on this basis the authors proposed three BHV-1 types which they designated I, II and III. The BHV-1 strain K-22 was isolated from a case of vulvovaginitis KENDRICK et al. (1958), and based on restriction endonuclease analysis, it is classified as strain III, MISR et al. (983).

Results of Bgl restriction enzyme as shown in (Table 1) were similar to those obtained by Eco $R_1$ but the difference was occurred in the migration pattern of the bands.

The restriction endonuclease Hind III cleaved the DNA of isolates (AH 28/88, AH 30/88, AH 31/88, AA 318/88, AA 321/88, AA 322/88, AA 366/88, AB 401/88, AA 406/88, AA 416/88, AA 428/88, AA 434/88, AA 452/88, AH 455/88, AH 467/88 and AH 470/88) as well as the DNA of Paramon reference strain into 10 fragments and the DNA of isolates (ST 143/88, ST 156/88, ST 177/88, AH 198/88, AH 205/88 as well as the DNA of Colorado reerence strain into 11 fragments as shown in (Table 1). Our results are inagreement and support the findings of MISRA et al. (1983) who found that therestriction endonuclease Hind III cleaved the BHV-1 DNA into 11 high molecular weight fragments ($13/10^6$ to $2x10^6$) and 3 to 4 low molecular weight fragments (less than $1x10^6$). They also found that on the basis of the Eco $R_1$ and Hind iii generated patterns, the BHV-1 isolates could be categorized into three main strains, I, II and III. Depending on the size fragment, Eco $R_1$-C strain-I could be further divided into sub-strains $I_1$ and $I_2$ and strain III into sub-strains $III_1$ and $III_2$. The same results were obtained by MAYFIELD et al. (1981) who found that BHV-I (Cooper strain) DNA was digested and fragmented into eleven fragments by Hind III restriction enzyme. By this enzyme the Hungerian reference strain DNA was cleaved into 8 fragments only.

The restriction endonuclease Bam HI cleaved the DNA of isolates (ST 143/88, ST 156/88, ST 177/88, AH 198/88, AH 205/88 and AA 352/88) as well as the DNA of Colorado reference strain into 9 fragments as shown in (Table 1). These results were confirmed thefinding of MAYFIELD et al. (1983) who found that this enzyme cleaved the DNA of BHV-1 (Cooper strain) into 9 fragments. Our results also showed that the same enzyme cleaved the DNA of isolates (AH 28/88, AH 30/88, AH 31/88, AA 318/88, AA 321/88, AA 322/88, AA 366/88, AB 401/88, AA 406/88, AA 416/88, AA 428/88, AA 434/88, AA 452/88, AH 455/88, AH 455/88, AH 467/88 and AH 470/88) as well as the Paramon reference strain into 8 fragments, while the DNA of Hungerian reference strain was cleaved into only 4 fragments. From data shown in (Table 1) our results seemed to be 6 of the isolates were related to Colorado strain and the other 16 isolates were related to Paramon reference strain. Non of the isolates was

related to the Hungerian reference strain (KK).

The prevalence of IBR virus related to Colorado strain in Tahta farm, may be attributed to the previous vaccination with living attenuated vaccines before importation. The virus remains latent for such long time till become apparent (HOMAN et al., 1980). In the mean time, it is worth to mention that all the Colorado related strains were isolated from respiratory or conjunctival infection.

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Table (1): Local and reference BHV-1 isolates, types of restriction enzyme, types of swab and number of fragments given by these enzymes.

| Local and reference Serial No. | Isolate identification code | Type of swab | Eco $R_1$ | BgI II | Hind III | Bam HI |
|---|---|---|---|---|---|---|
| | | | Fragments | Fragments | Fragments | Fragments |
| 1 | AH 28/88 | V.S. | 6 | 6 | 10 | 8 |
| 2 | AH 30/88 | V.S. | 6 | 6 | 10 | 8 |
| 3 | AH 31/88 | V.S. | 6 | 6 | 10 | 8 |
| 4 | ST 143/88 | N.S. | 7 | 7 | 11 | 9 |
| 5 | ST 156/88 | N.S. | 7 | 7 | 11 | 9 |
| 6 | ST 177/88 | C.S. | 7 | 7 | 11 | 9 |
| 7 | AH 198/88 | N.S. | 7 | 7 | 11 | 9 |
| 8 | AH 205/88 | N.S. | 7 | 7 | 11 | 9 |
| 9 | AA 318/88 | V.S. | 6 | 6 | 10 | 8 |
| 10 | AA 321/88 | V.S. | 6 | 6 | 10 | 8 |
| 11 | AA 322/88 | V.S. | 6 | 6 | 10 | 8 |
| 12 | AA 352/88 | N.S. | 7 | 7 | 11 | 9 |
| 13 | AA 366/88 | V.S. | 6 | 6 | 10 | 8 |
| 14 | AB 401/88 | V.S. | 6 | 6 | 10 | 8 |
| 15 | AA 406/88 | V.S. | 6 | 6 | 10 | 8 |
| 16 | AA 416/88 | V.S. | 6 | 6 | 10 | 8 |
| 17 | AA 428/88 | V.S. | 6 | 6 | 10 | 8 |
| 18 | AA 434/88 | V.S. | 6 | 6 | 10 | 8 |
| 19 | AA 452/88 | V.S. | 6 | 6 | 10 | 8 |
| 20 | AH 455/88 | V.S. | 6 | 6 | 10 | 8 |
| 21 | AH 467/88 | V.S. | 6 | 6 | 10 | 8 |
| 22 | AH 470/88 | V.S. | 6 | 6 | 10 | 8 |
| Colorado | | - | 7 | 7 | 11 | 9 |
| Paramon | | - | 6 | 6 | 10 | 8 |
| Hungerian | | - | 5 | 5 | 8 | 4 |

*Eco* $R_1$

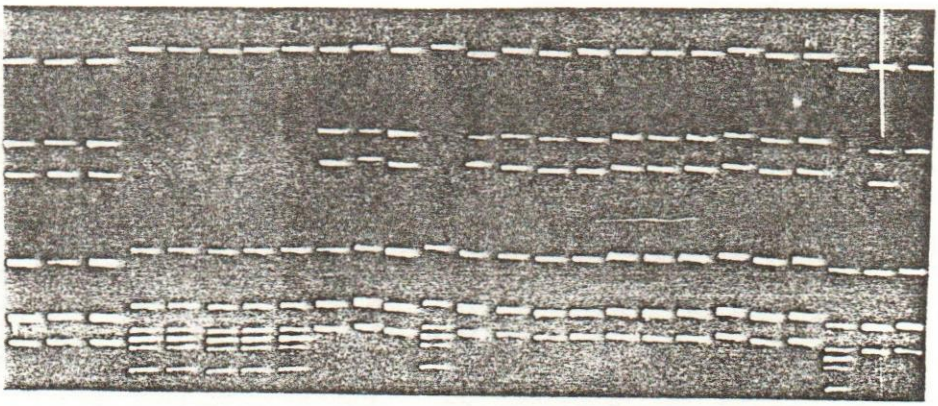
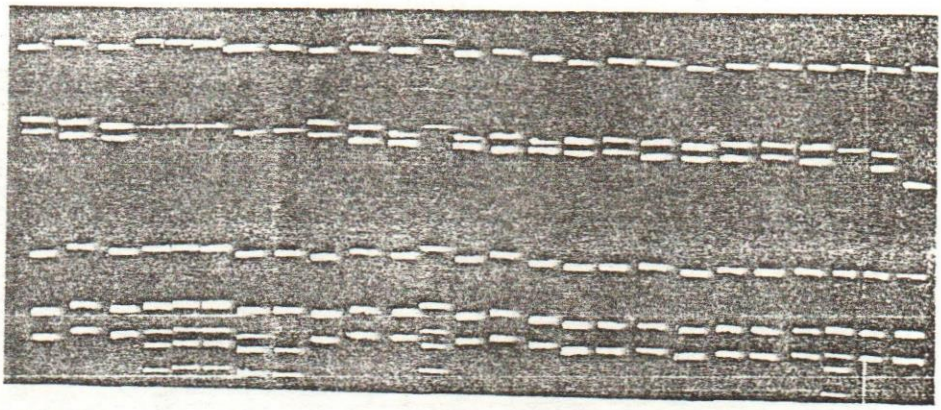


Fig. 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 C P K K

**Fig. (1 a):** Effect of Eco $R_1$ digests on local BHV-1 and reference strains DNA.

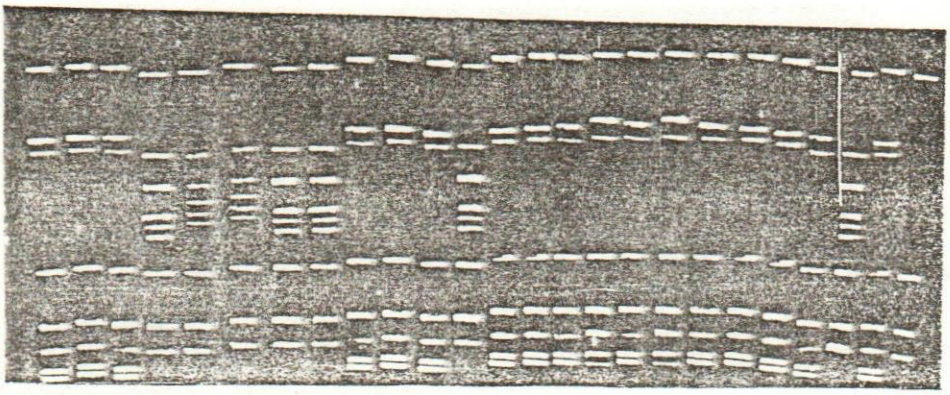
*BgI*



1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 C P K K

**Fig. (1 b):** Effect of BgI digests on local BHV-1 and reference strains DNA.

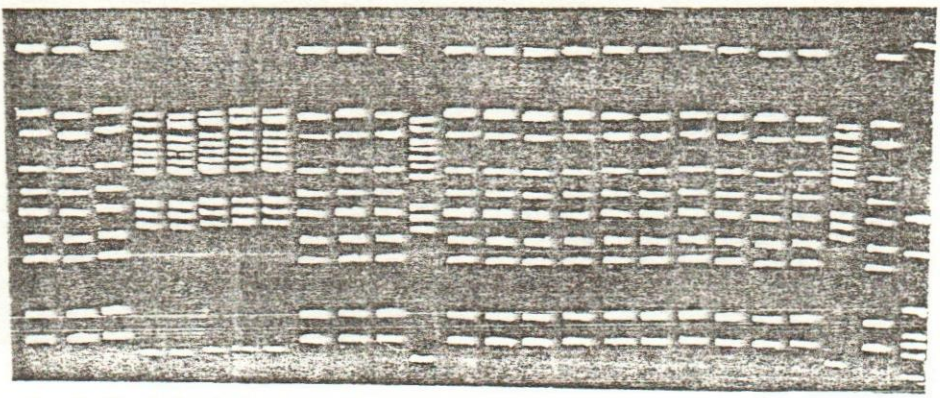
*Bam HI*



1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 C P KK

**Fig. (1 d):** Effect of Bam HI digests on local BHV-1 and reference strains DNA.

*Hind III*



1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 C P K

**Fig. (1 c):** Effect of Hind III digests on local BHV-1 and reference strains DNA