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STUDIES ON RINDERPEST LIKE DISEASES
AND THEIR CAUSES
(With One Table and One Figure)

By

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SUMMARY

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

These isolates were compared with reference strains of IBRV (Colorado, Paramon & Hungarian strains) using restriction endonuclease enzymes, (Eco R1, Bgl II, Bam HI and Hind III).

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

No isolate was related to the Hungarian 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 a molecular 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, meningecephalitis and generalized systemic infections (GIBBS and REWEYEMAMU, 1977).

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

Many attempts have been made to differentiate strains of infectious bovine rhinotracheitis, especially those of respiratory and genital 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 deoxyribonucleic 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 & GRATZK, 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 (GRTEK et al., 1966, BUENING & GRATZK, 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 genital and Respiratory & conjunctival tracts of cattle with the international reference strains (Colorado & Hungarian) 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 kindlely 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. Hungarian 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).

RINDERPEST LIKE DISEASES

RESULTS

Restriction endonuclease analysis of viral DNA:

Figure (1a) 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 isolates 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 Hungarian strain was cleaved into 5 fragments. Also the restriction endonuclease enzyme Bgl gave the same results obtained by Eco R1 but differed in the migration patterns (Fig. 1b).

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 11 and 10 fragments respectively. But its cleavage to the DNA of the Hungarian strain lead to 8 fragments (Fig. 1c). 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 Hungarian strains into 9, 8 and 4 fragments respectively (Fig. 1d). 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 Hungarian 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 prominent 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).


Hungarian reference strain was cleaved into only five fragments. Our results are in
de agreement and extended the findings of MAYFIELD et al. (1983) who found that the
purified Cooper strain DNA was cleaved with restriction. Eco R₁ 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 R₁ cleaved BHV-1 DNA into six or seven
fragments, also found in their analysis of 116 BHV-1 isolates, that differences in clea-
vage patterns were not associated with different disease syndrome. Three cleavage
patterns were found among the 116 isolates, 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 endo-
nuclease 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 R₁ 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,
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 reference strain into 11 fragments as shown in (Table
1). Our results are in agreement and support the findings of MISRA et al. (1983) who
found that the restriction endonuclease Hind III cleaved the BHV-1 DNA into 11 high
molecular weight fragments (13/10⁶ to 2x10⁶) and 3 to 4 low molecular weight frag-
ments (less than 1x10⁶). They also found that on the basis of the Eco R₁ 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 R₁-C strain-I could be further divided
into sub-strains 1, and I₁ and III into sub-strains III₁ and III₂. The same results
were obtained by MAYFIELD et al. (1981) who found that BHV-1 (Cooper strain) DNA
was digested and fragmented into eleven fragments by Hind III restriction enzyme.
By this enzyme the Hungarian 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 the finding 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,
well as the Paramon reference strain into 8 fragments, while the DNA of Hungarian
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

RINDERPEST LIKE DISEASES

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 impor-
tation. 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.

REFERENCES

Bagust, T.J. (1972): Comparison of the biological, biophysical and antigenic properties
of four strains of IBR. J. Comp. Path., 82, 356-374.


Black, D.V. and Slack, g. (1972): A comparison of two strains of infections bovine rhino-

Buening, G.M. and Gratzek, J.B. (1967): Comparison of selected characteristics of four

Crandell, R.A. (1972): Isolation of infectious bovine rhinotracheitis virus from feaces

rhinotracheitis virus DNA. Internat. Conference on Human Herpes viruses, Atlanta,
341-354.


infectious pustular vulvovaginitis virus infectious bovine rhinotracheitis. Cornell

Gratzak, J.B.; Jenkins, R.A.; Peter, C.P. and Ramsey, F.K. (1966): Isolation and charac-
terization of a strain of infectious bovine rhinotracheitis virus associated with
enteritis in cattle: Comparative development study by fluorescent antibody tracing


431-453.


Liess, Von B.; Knocke, K.W. and Schimmelpfennig, H. (1960): Feststellung der virus beding-
ten Rhinotracheitis infectiosa des Rindes. II Kulturelle, serologische und elektronen-
optische charakterisierung des virus unter Berucksichtigung der in Gewebekulturen
auftretenden zytopathischen Veranderungen. Dtshe. Tierarztl. Wochenschr.; 67:
551-558.


### RINDERPEST LIKE DISEASES

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

<table>
<thead>
<tr>
<th>Local and reference</th>
<th>Type of swab</th>
<th>Eco R&lt;sub&gt;1&lt;/sub&gt;</th>
<th>Bgl II</th>
<th>Hind III</th>
<th>Bam HI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serial Isolate No.</td>
<td>Type of swab</td>
<td>Fragments</td>
<td>Fragments</td>
<td>Fragments</td>
<td>Fragments</td>
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<td>Identification code</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>1 AH 28/88</td>
<td>V.S.</td>
<td>6</td>
<td>6</td>
<td>10</td>
<td>8</td>
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<tr>
<td>2 AH 30/88</td>
<td>V.S.</td>
<td>6</td>
<td>6</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>3 AH 31/88</td>
<td>V.S.</td>
<td>6</td>
<td>6</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>4 ST 143/88</td>
<td>N.S.</td>
<td>7</td>
<td>7</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>5 ST 156/88</td>
<td>N.S.</td>
<td>7</td>
<td>7</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>6 ST 177/88</td>
<td>C.S.</td>
<td>7</td>
<td>7</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
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<td>N.S.</td>
<td>7</td>
<td>7</td>
<td>11</td>
<td>9</td>
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<tr>
<td>8 AH 205/88</td>
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<td>7</td>
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<td>5</td>
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<tr>
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<td>5</td>
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<tr>
<td>11 AA 322/88</td>
<td>V.S.</td>
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<td>6</td>
<td>10</td>
<td>5</td>
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<td>12 AA 352/88</td>
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<td>6</td>
<td>10</td>
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<td>14 AB 401/88</td>
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<td>6</td>
<td>10</td>
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<tr>
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<td>6</td>
<td>10</td>
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<tr>
<td>16 AA 416/88</td>
<td>V.S.</td>
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<td>4</td>
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<td>8</td>
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<tr>
<td>17 AA 428/88</td>
<td>V.S.</td>
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<td>6</td>
<td>10</td>
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<tr>
<td>18 AA 434/88</td>
<td>V.S.</td>
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<td>6</td>
<td>10</td>
<td>8</td>
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<tr>
<td>19 AA 452/88</td>
<td>V.S.</td>
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<td>6</td>
<td>10</td>
<td>8</td>
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<tr>
<td>20 AH 455/88</td>
<td>V.S.</td>
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<td>10</td>
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<tr>
<td>21 AH 467/88</td>
<td>V.S.</td>
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<td>6</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
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<td>8</td>
</tr>
<tr>
<td>Colorado</td>
<td>-</td>
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<td>7</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>Paramon</td>
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<td>6</td>
<td>10</td>
<td>8</td>
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<td>Hungarian</td>
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<td>5</td>
<td>5</td>
<td>8</td>
<td>4</td>
</tr>
</tbody>
</table>

Eco $R_1$

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

Bgl

Fig. (1 b): Effect of Bgl digests on local BHV-1 and reference strains DNA.
RINDERPEST LIKE DISEASES

Bam HI

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

Hind III

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