Isolation of Bovine Herpesvirus type 1 from bovine semen in Turkey

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SUMMARY
In this study, semen samples from sixty bulls were used for virus isolation into sensitive cell culture. Five viruses were isolated by inoculation of 60 semen samples into the cell cultures. The isolated viruses were identified as BHV-1 according to results of cross microneutralization test by using hyperimmune sera from rabbits. Obtained data showed that BHV-1 may exist in the semen, and sensitive cattle may be infected by virus via insemination.

KEY-WORDS : Bovine Herpesvirus type 1 - cattle - semen.

1. Introduction
Viruses may survive in semen from animals with infection and stored for artificial insemination. The infected semen may be distributed to throughout country and may also exported to other countries. Thus, virological examination of both donor bulls and their semen are extremely important in insemination centres.

Bovine Herpesvirus type 1 (BHV-1) may be isolated from semen of acute or latent infected bulls [1, 15, 19]. Semen from infected bulls are act as a vehicle to spread to wide area through artificial insemination. Bulls with antibodies to BHV-1 are accepted epidemiologically reservour and some authors [2, 5, 23, 27] reported that BHV-1 was isolated from semen (stored at low temperatures) from bulls in artificial insemination centres. BURGU and AKÇA [4] detected neutralizing antibodies against BHV-1 in blood sera obtained from bulls in artificial insemination centre in Turkey. But, BHV-1 isolation from bull’s semen has not been reported in Turkey until present.
2. Materials and Methods

A) ANIMALS

Sixty semen samples were obtained from 29 bulls in artificial insemination center in Ankara and 31 bulls used for natural breeding in private farms in Konya. Bulls were 3-10 years old.

B) VIRUS ISOLATION

Semen samples were prepared according to method described by LOWEN and DARCEL [16]. Briefly, 0.2 ml of each semen specimen (1:10 dilution) was inoculated into duplicate tubes of foetal calf kidney (FCK) cell culture. After incubation for 1 hour at 37°C, the monolayer cell cultures were washed three times with PBS and Eagle’s Minimum Essential Medium (EMEM) was added. Cell culture tubes were incubated at 37°C and were controlled daily for cytopathologic effect (cpe) by inverted microscope. After seven days, cell culture medium was harvested by freeze-thawing and centrifuged to remove cellular debris at 4°C for 30 minutes at 3000 rpm/min. The obtained supernatants were used for three blind passages onto FCK cell cultures and then supernatants obtained from cell culture tubes with cpe were used for virus identification.

C) VIRUS AND HEPERIMMUN ANTISERA

Colorado reference strain of BHV-1 was used to prepare hyperimmum antisera. The hyperimmum antisera to BHV-1 were collected from two healthy New Zealand rabbits by multiple injections of virus. These rabbits were scan against BHV-1 before the virus was inoculated. After 7 days following last virus inoculation, blood was taken from heart of rabbits and sera were obtained. After sterility assay, antibody titer of hyperimmum antisera were detected as reported by FREY and LIESS [11] and stored at —20°C until used for identification of virus by neutralization test.

D) NEUTRALIZATION TEST

Neutralization test was done between isolates and hyperimmun antisera to BHV-1 Colorado strain. The hyperimmun antisera from rabbits and each isolate diluted at 100 CCID50 were subjected to cross mNT. The results were read for cpe by inverted microscope.

3. Results

Titer of BHV-1 Colorado strain was calculated as 10^-6.75 CCID50/0.05 ml according to KAERBER [13]. SN50 titer of undiluted hyperimmun antisera which was detected positive by means of BHV-1 antibodies was determined as 1/32.

During this study, semen samples from sixty bulls were done three consecutive passages onto FCK cell culture. Five samples produced cpe on second or third passages on FCK cell culture. In result of inoculation onto cell culture of the other semen samples, no viral agent was detected (Table I). But we did not investigate for noncytopathogen (ncp) viruses in semen samples in this study.

Three out of five isolates were identified as BHV-1 by mNT which was done between isolates and hiperimmun antisera against BHV-1.

4. Discussion

Prepared semen in artificial insemination centers are stored at -196°C and sent to other places when its necessary. After freezing procedure, stability of viruses in contaminating semen causes transmission of virus among cattle populations. The contamination of semen with microorganisms can be prevented when antimicrobial agents were added during prepared semen for insemination. Unfortunately, these antimicrobial agents have not affected on viruses in semen. Because of this reason, bulls used for insemination should be periodically tested for viruses in suitable laboratories, particularly to prevent from risk of outbreak of viruses-induced diseases.

It has been reported that numerous viruses were isolated from semen [3, 7, 12, 16, 17, 18, 21, 22, 30]. BHV-1 that also caused infertility problems in herds attracts attention among these viruses. BHV-1 infection transmits from subclinic or latent infected animals to healthy animals [25]. The virus in latent infected animals is occasionally reactivated with stress factors such as pregnancy, transport, vaccination and appli-
cation of corticosteroid. Therefore, all of latent infected animals are called virus reservoir [8, 20]. Because of semen from acute, subclinical or latent infected bulls are stored in liquid nitrogen until used, they cause to spread of infection by artificial insemination. So it is also accepted that seropositive bulls are epidemiologically porter and play an important role for distribution of the virus [2, 15]. Therefore, bulls must be seronegative against BHV-1 infection before semen are taken [15].

ELAZHARY et al. [10] isolated BHV-1 from semen samples from a donor bull used for insemination in a management, which had infertility and abort problems, by using immunofluorescence test. They also carried out isolation of virus from uterus secretions of inseminated cows by the bull and reported that semen used for insemination plays an important role for distribution of BHV-1 infection. BHV-1 was isolated from nasal, conjunctival fluid, preputial-washing fluid and semen samples but vaginal fluid obtained from cattle with respiratory and genital tract disease in Israel by ABRAHAM et al [1]. The same researchers also stated that neutralizing antibodies against the virus were detected in blood sera of all animals affected from infection in this herd. COLLERY et al [6] isolated IBR/IPV virus from two vaginal swap and one preputial-washing fluid from a herd including 14 cows and one bull which they had infertility problems. In this study, BHV-1 was isolated from semen samples of five bulls. One out of five animals was in an artificial insemination center and others were in private farms.

Immunoperoxidase (IP), reverse haemagglutination (RPHA), immunofluorescence (IF), ELISA test, Polymerase chain reaction (PCR), experimental animals and cell cultures are commonly used for isolation and identification of BHV-1 [9, 10, 24]. BHV-1 might be propagated into foetal calf kidney, lung, turbinate, skin foetal lung and MDBK cell cultures [20, 28]. SINGH et al [26] reported that FCK and turbinate cell cultures were more sensitive than MDBK cell culture for first isolation of virus during their studies on BHV-1 isolation. Althought the cell culture technique is used for BHV-1 isolation widely, KAHRS et al [14] suggested that this technique is an expensive and time-consuming method. On the other hand VAN ENGELENBURG et al [29] reported that PCR –although expensive- is an alternative test to isolation techniques, because the results could be obtained in only one day but this period takes at least seven days in the cell culture methods.

The toxic effect of semen on cells in cultures was regarded as another disadvantage of cell culture technique, this toxic effect could be overcome by diluting of semen [16]. Besides, researchers stated that the toxic effect in cell cultures may be observed like cpe and three blind passages of semen samples should be done into cell culture. In this study, cell culture technique was used for virus isolation from semen and semen samples were diluted (1:10 ratio) to prevent from their toxic effect on cell cultures.

BHV-1 infections take an important place among different infections causing infertility problems of cattle breeding. The presence of both seropositive and virus-shedding animals in cattle populations according to results of our study and other studies show that BHV-1 infection is also very important problem for Turkey. So, bulls used for artificial and natural insemination should be checked for the virus in semen, preputial washing-fluid, leukocyte, nasal and conjunctival fluid and controlled serologically in regular periods.

Bibliography


