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A COMPARATIVE EVALUATION of BOVINE HERPESVIRUS–1 INFECTION by ENZYME LINKED IMMUNOSORBENT ASSAY and SERUM NEUTRALIZATION TEST in KONYA PROVINCE*

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KONYA bölgesindeki sığırlarda bovine herpesvirus–1 enfeksiyonunun nötralizasyon testi ve enzyme linked immunosorbent assay ile karşılaştırmalı olarak değerlendirilmesi

ÖZET

Bu çalışmada, Konya KONET mezbahasına kesim amacıyla getirilen etçi sığırlardan 100 adet kan örneği toplanmıştır. 100 adet kan örneğinden elde edilen serum örneklerinde sığır herpesvirus tip 1'e (BHV–1) karşı oluşan serum antikorlarının belirlenmesi amacıyla, Enzyme-linked immunosorbent assay (ELISA) ve serum nötralizasyon (SN) test teknikleri kullanılmıştır. ELISA ve SN test tekniklerinin, BHV-1'e karşı oluşan serum antikorlarını belirleme etkinlikleri değerlendirilmiştir. ELISA sonuçlarına göre 19 (%19) pozitif, 81 (%81) negatif sonuç tespit edilmiştir. SN testi ile 13 (%13) pozitif serum ve 87 (%87) negatif serum tespit edilmiştir. İki serolojik metoda ait spesifite ve sensitivite değerlerinin karşılaştırılması sonucunda BHV-1'e karşı oluşan serum antikorlarının tespitinde ELISA tekniğinin SN testine göre daha fazla duyarlılık gösterdiği belirlenmiştir.

ANAHTAR KELİMELER: ELISA, BHV–1, SN.

SUMMARY

In this research, one hundred (100) blood samples were collected from beef cattle that was brought for the purpose of slaughter in KONET slaughterhouse located in Konya. Enzyme-linked immunosorbent assay (ELISA) and serum neutralization (SN) test techniques were used for the detection of serum antibodies to bovine herpesvirus 1 (BHV–1) on serum samples seperated from 100 blood samples. ELISA and SN test were evaluated for their ability to detect serum antibodies to BHV–1. Nineteen (19%) serum samples were detected to be positive and 81 (81%) serum samples were detected to be negative by ELISA. Thirteen (13%) serum samples were detected as positive and 87 (87%) serum samples were detected as negative by SN test. At the end of the comparison of the sensitivity and specifity values of the two serological methods, ELISA technique showed more sensitivity than the SN test for the detection of serum antibodies to BHV-1.

KEY WORDS: ELISA, BHV–1, SN.

*This paper was summarized from the master thesis.

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INTRODUCTION

Bovine Herpesvirus 1 (BHV–1) is classified in Alphaherpesvirinae subfamily and Herpesviridae family. BHV-1 is the causative agent of Infectious Bovine Rhinotracheitis (IBR), Infectious Pustular Vulvovaginitis (IPV) or balonoposthitis (IPB), which is associated with several diseases in cattle, including conjuctivitis, abortion, meningoencephalitis, enteritis and a generalized infection in young calves. BHV-1 (also known as IBR virus) infection usually affects the respiratory or genital tracts. BHV–1 infection causes great economic loses including abortions, early embryonic and fetal deaths, which result in infertility, decreased milk production and weight loses in dairy herds (Bolat and Kandil 1990, Barwinek et al. 1997). BHV-1 infection can show latency in the neuronal ganglia after the primary infection and latently infected cattle can be a virus source for noninfected animals in the herd (Ackermann and Wyler 1984). So, the early detection of seropositive animals is important to eliminate latent animals from the herd.

BHV-1 has a high prevalance among beef cattle among the other countries as in Turkey. Serum neutralization (SN) test and enzyme-linked immunosorbent assays (ELISAs) are routinely used for BHV–1 antibody detection among beef cattle by various researchers (Riegel et al. 1987, Bolat and Kandil 1990, Lyaku et al. 1990, Cerqueira et al. 2000).

In this research, blood samples of 100 beef cattle were collected from the KONET slaughterhouse to detect the serum antibodies of BHV–1 by ELISA and SN test techniques. It was aimed to evaluate the sensitivity and specifity of two serological methods (ELISA and SN test).

MATERIALS and METHODS

Animals: Blood samples of 100 beef cattle (between 1 and 2 years old) which had been collected from the KONET slaughterhouse were used to determine the diagnostic sensitivity and specificity of the SN test and ELISA.

Serum samples: The blood samples were stored overnight at 4^0C and the serum were removed after centrifugation at 2500–3000 rpm for 30 minutes. All serum were heat inactivated at 56 ^{0}C for 30 minutes. Each serum sample was stored in small quantity at -20 ^{0}C after sterilization with membrane filtration method.

Virus

IBR/IPV Colorado reference strain was delivered from Selcuk University Faculty of Veterinary, Department of Virology. Virus was frozen at -80 ^{0} C until processed.

Madin Darby Bovine Kidney (MDBK) cell culture was used for virus titration and SN tests (Figure 1). The virus was succesfully grown in MDBK cell culture in Dulbecco's Modified Eagle's Medium (DMEM) with the supplement of 10% inactive fetal bovine serum and was incubated at 37 ^{0}C. Virus was added to cell culture and incubated for one hour at 37 ^{0}C. At the end of the incubation period. DMEM was supplemented to the cell suspension and reincubated at 37 ^{0}C. After 48–72 hours, it was controlled by inverted microscope for the presence of the cytopathic effect (CPE) (Figure 2).

The virus titer was detected as $TCID_{50}$ $10^{-5,75}$/0,1 ml according the Kaerber method (1964) at the end of the third day. This titration unit was used in SN test.

SN test

Virus neutralizing antibody assay was carried out using the modified serum microneutralization test described by Frey and Liess (1971). 96- well flat – bottom microtitration test plates was used for each serum sample. Approximately 0.05 ml (100 $TCID_{50}$: $10^{-3,45}$ /0.05 ml) of the BHV-1 strain was used. 0.05 ml DMEM was supplied to each cell of the 96- well flat-bottom plates. Four-well was choosed for virus control and 0.05 ml DMEM and diluted virus (0.05 ml) was added to virus control wells. Another four-well was choosen for cell control and 10% inactive fetal bovine serum supplemented 0.1 ml DMEM was added. The plate was covered with a nontoxic adhesive band and incubated for one hour at 37 ^{0}C in a CO_2 incubator. After the incubation period, adhesive band was removed and 0.05 ml MDBK cell suspension (200.000 cell/ml) was added to each well. The plates were covered with a nontoxic band again and incubated during three days at 37 ^{0}C. At the end of the third day, plates were controlled for the presence of the CPE on virus control wells by inverted microscope. At the and of the test serum neutralization titer was expressed according to the method of Kaerber (1964).

ELISA

To detect the presence of the spesific antibodies for BHV-1, Infectious Bovine Rhinotracheitis ELISA (serum) commercial test kit was used according to the manufacturer's recommendations.

RESULTS and DISCUSSION

One hundred (100) serum samples were used for comparison of the ELISA with the SN test. Table shows the results of the two serological methods that were used to determine BHV–1 serum antibodies in collected blood samples. Nineteen (19%) of serum samples were detected to be positive and 81 (81%) serum samples were detected to be negative by ELISA. Thirteen (13%) of serum samples were detected as positive and 87 (87%) serum samples were detected as negative by SN test. A similar

comparative study of the two tests indicated that the ELISA detected more seropositive animals (61.6%) than the standard serum neutralizing test (49.9%) and ELISA was considered to be technically superior as a routine diagnostic test for the detection of infectious bovine rhinotracheitis viral antibody in bovine serum (Cho and Bohac 1985).

A general picture of the prevalence of BHV–1 infection in several countries on three continents has been given in a review by Straub (1990). For the countries quoted the prevalences of seropositive cattle varied from 14,3–60% in Africa, 36,6–48% in Central and South America and 5,6-76,1% in Europe.

In this study, a low seropositivity was observed to BHV-1 (13-19%) among beef cattle in Konya region. Various researchers obtained similar results in Turkey as it was detected in this research (Erhan et al. 1971, Özkul et al. 1995, Ünver 2002). On the other hand, some previous studies were reported higher antibodies against IBR than our study among beef cattle in Konya region (Öztürk et al. 1988, Yavru et al. 1998, Yavru et al. 2005).

Compared with SN test, ELISA showed 84,6% sensitivity and 90,8% Specificity in the present study (Table).

Boelaert et al. (2000) were obtained ELISA and SN test sensitivities and spesificities 70–99% and 90-99.7%, respectively. Marjorie et al (2001), were explained 92.37% sensitivity and 92.56% specificity between ELISA and SN tests.

In this research, altough the presence of the low number of serum samples, the results indicated that the ELISA is superior to the SN test to detect serum antibodies of BHV-1. Control programmes against IBR infection is necessary in all over the world as in our country. Differences in prevalences between regions and countries could be explained by factors such as herd size, disease control, type of breeding and age of the animal (McDermott et al. 1997).

Based on the results, it was observed that the ELISA is superior to the SN test which is the official serological test for the detection of antibodies to BHV-1. This test was shown to be a suitable alternative to SN test in the detection of serum antibodies to BHV–1 in beef cattle. In this research, low seroprevalence observed to (BHV-1) can be explained by low number of sample. More information is needed to better understanding of the herd-level risk factors that enhance the transmission of (BHV-1) between different herds, under the management and ecological conditions of Konya.

Table. Comparison of ELISA with SN test on beef cattle serum samples.

ELISA reaction	Serum neutralization		Total	Sensitivity (%)	Specificity (%)
	Positive	Negative			
Positive	11	8	19	84,6	90,8
Negative	2	79	81		
Total	13	87	100		

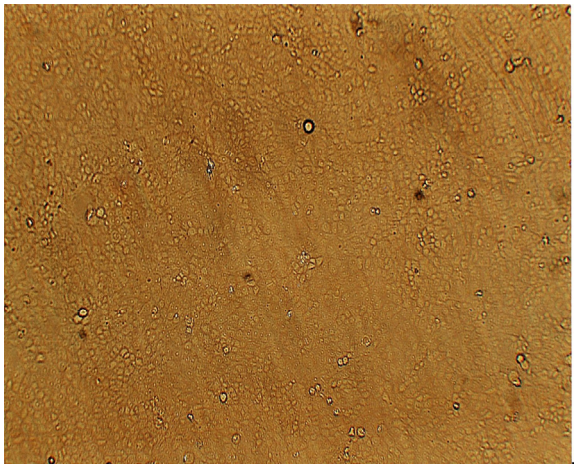

Figure 1. MDBK cell culture (cell control) (x10).

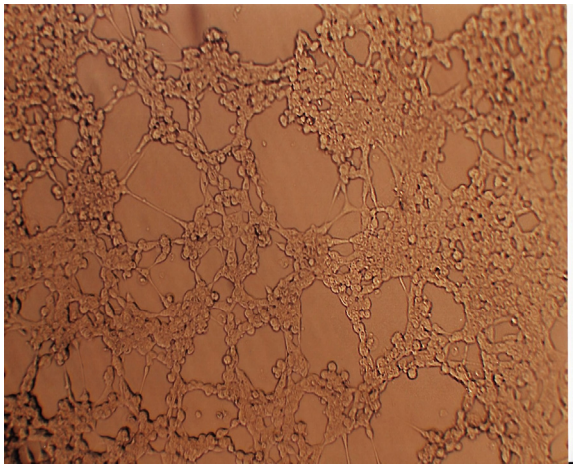

Figure 2. Cythopathologic effect (CPE) after three days (MDBK cell culture infected with IBR/IPV Colorado reference strain) (x10).

REFERENCES

Ackermann M, Wyler R (1984) The DNA of an IPV strain of bovid herpesvirus 1 in sacral ganglia during latency after intravaginal infection. Vet Microbiol; 9: 53-63.

Barwinek F, Taylor NM, Tatar N (1997) Prevalance of antibodies against BVDV, BHV-1 and BVLV in cattle of Turkish villages and implications for the governmental veterinary services. Viehl and Fisch; 32: 421–428

Boelaert F, Biront F, Soumare B, Dispas M, Vanopdenbosch E, Vermeersch JP, Raskin A, Dufey J, Berkvens D (2000) Prevalence of bovine herpesvirus-1 in the Belgian cattle population, Prev Vet Med; 45: 285-295.

Bolat Y, Kandil M (1990) Sığırların enfeksiyöz rhinotracheitis (IBR) virus enfeksiyonu üzerine serolojik araştırma. Fırat Üniv Sağ Bil Derg; 4: 105-110.

Cerqueira RB, Carminati R, Silva JM, Soares GC, Meyer R, Sardi S (2000) Serological survey for Bovine Herpesvirus 1 in cattle from different regions in the state of Bahia, Brazil Braz J Vet Res Anim Sci; 37:6.

Cho HJ, Bohac JG (1985) Sensitivity and specificity of an enzyme-linked immunosorbent assay for the detection of infectious bovine rhinotracheitis viral antibody in cattle. Can J Comp Med; 49(2): 189–194.

Erhan M, Onar B, Csontos L, Hopkins I G (1971) Serological survey on some virus and bedsonia diseases of cattle. Pendik Vet Kont Arş Derg; 4: 55-58.

Frey HR, Liess B (1971) Vermehrungskinetik und vermendbarkeit eines stark zytopatogenen VD-MD virusstammes für disgnostische untersuchungen mit der mikrotiter-methode. Zbl Vet Med B; 18:61–71.

Kaerber G (1964) In diagnostic procedures for virus and rickettsial disease. Public Health Ass; 3: 48–50.

Lyaku JRS, Nettleton PF, Scott GR (1990) A quantitative enzyme-linked immunosorbent assay for Bovine Herpesvirus Type 1. Vet Microbiol; 18 (3): 199–205.

Marjorie FB, Paulo AE, Cynthia SS, Spilki FR, Silva TC, Dotta MA (2001) ELISA de bloqueio monoclonal para o diagnóstico sorológico de infecções pelo herpesvírus bovino tipo 1 (BHV–1). Pesq Vet Bras; 21:33–37.

McDermott JJ, Kadohira M, O'Callaghan CJ (1997) A comparison of different models for assessing variations in the sero-prevalence of infectious bovine rhinotracheitis by farm, area and district in Kenya. Prev Vet Med; 31:147–150.

Özkul A, Çabalar M, Bilge S, Akça Y, Burgu İ (1995) Süt sığırcılığı işletmelerinde rastlanan IBR/IPV ve BVD virus infeksiyonlarının infertilite olgularındaki rolü. Ankara Üniv Vet Fak Derg; 42: 381–387.

Öztürk F, Toker A, Yavru S (1988) Konya hayvancılık merkez araştırma enstitüsü sığırlarında enfeksiyöz bovine rhinotracheitis-enfeksiyöz pustular vulvovaginitis (IBR/IPV) üzerine araştırmalar. Selçuk Üniv Vet Fak Derg; 4:53–64.

Riegel CA, Ayers VK, Collins JK (1987) Rapid, sensitive, competitive serologic enzyme-linked immunosorbent assay for detecting serum antibodies to Bovine Herpesvirus type 1. J Clin Microbiol; 25: 2418-2421.

Straub OC (1990) Virus infections of ruminants. Edited by Z. Dinter, B. Morein, Department of Veterinary Microbiology, Section of Virology, Uppsala, Sweden.

Ünver A (2002) Sığırlarda BHV-1 enfeksiyonlarının PZR ve ELISA ile saptanması ve risk faktörlerinin belirlenmesi (Doktora Tezi), İstanbul Üniv Sağ Bil Enst, Mikrobiyoloji AB.

Yavru S, Şimşek A, Öztürk F (1998) Boğalarda Bovine Herpesvirus Tip 1 (BHV–1) enfeksiyonunun serolojik ve virolojik olarak araştırılması. Selçuk Üniv Vet Bil Derg; 14:101–110.

Yavru S, Şimşek A, Yapkıç O, Kale M (2005) Serological evaluation of viral infections in bovine respiratory tract. Acta Vet Beograd; 55:219–226.