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SEROSURVEY ON BORDER DISEASE VIRUS INFECTION IN SHEEP AND GOATS IN AL-AHSA REGION, KINGDOM OF SAUDI ARABIA

(With 3 Tables)

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مسح سيرولوجى للعدوى بفيروس مرض البوردر فى الإغنام والماعز في منطقة الأحساء بالمملكة العربية السعودية

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تم عمل مسح سيرولوجى للكشف عن الأجسام المضادة لفيروس مرض البوردر في الأغنام والماعز في منطقة الأحساء بالمملكة العربية السعودية فى الفترة 2006-2007 حيث تم تجميع 1471 عينة سيرم من الأغنام و الماعز وتم فحصهم باختبار الأليزا المتوفر تجاريا. اظهرت النتائج نسبة اصابه 72,23 % و 11,13 % فى الأغنام (n= 548) والماعز (n=923) على الترتيب. أوضح التحليل الأحصائى أن هناك علاقة قوية لتاثير العمر على معدل الأصابة بالمرض. هناك فروق معنوية لقابلية الأغنام للعدوى بالمقارنة بالماعز ولا توجد فروق معنوية لتأثير الجنس فى الأغنام والماعز وتأثير السلالة فى الماعز على معدل الأصابة بفيروس مرض البوردر وكان هناك فروق معنوية لتأثير السلالة فى الأغنام على معدل الأصابة حيث ان سلالة الأوازى الاكثر قابلية للعدوى وسلالة البربرى الأقل قابلية للعدوى.نخلص من ذلك أن مرض البوردر من فيروسات البيستى متواجد بصورة خفية (سيرولوجية) في الأغنام والماعز في منطقة الأحساء بالمملكة العربية السعودية.

SUMMARY

A serological survey was conducted in attempt to detect the antibodies against border disease (BDV) in sheep and goats at AL-Ahsa region, Kingdom of Saudi Arabia. During the period 2006-2007, a total of 1471 serum samples were subsequently collected and serologically tested by using a commercial ELISA kit, which revealed that 23.72% and 13.11% of examined sheep (n=548) and goats (n=923) were seropositive to BDV infection, respectively. The offered data declare a strong correlation between the age of the tested animals and seroprevalence of BDV

infection. The data also revealed highly significant increase (P< 0.07) in the susceptibility rate of sheep to BVD infection than goats. There is no significant effect of sex. The effect of breed on goats is also non-significant, but breeds of sheep varies significantly in the serosurvey of border disease virus antibodies as the Awassi is the higher and the barbari is the lowerest. It can be concluded that BDV is serologically established in sheep and goats in Al-Ahsa region in Kingdom, Saudi Arabia.

Key words: *Border disease, virology, sheep, goats*

INTRODUCTION

The genus Pestivirus, Family Flaviviridae, comprises bovine viral diarrhea virus, classical swine fever virus, and border disease virus (Becher *et al.,* 1997). These viruses were classified primarily according to the host from which they were isolated. It is now known that cross-infection among species occurs readily. Consequently, these viruses are recently grouped more according to their reactivity with monoclonal antibodies and to their nucleotide sequences. Phylogenic analysis of antigenic similarities indicated the presence of seven major antigenic groups corresponding to BVDV-1, BVDV-2, CSFV, BDV-1, BDV-2, BDV-3 (Becher *et al.,* 2003), and one tentative strain (Giraffe 1) represented by a single strain (H138) isolated from a giraffe in Kenya (Avalos-Ramirez *et al.,* 2001).

Border disease (BD) is a congenital virus disease of sheep and goats first reported in 1959 from the border region of England and Wales. Distribution of the virus is worldwide. Ovine and caprine pesti viruses were isolated and genomic characterized from lambs and kids (Pratelli *et al.,* 2001; De Mia *et al.,* 2005; De Mia *et al.,* 2005 and Mishra *et al.,* 2007). Prevalence rates of BD virus antibodies vary in sheep from 5 to 50% between countries and characterized by abortion. Clinical signs in sheep include barren ewes, abortions, stillbirths and the birth of small weak lambs. Affected lambs can show tremor, abnormal body conformation and hairy fleeces ('hairy-shaker' or 'fuzzy' lambs). Vertical transmission plays an important role in the epidemiology of the disease. Infection of fetuses can result in the birth of persistently infected (PI) lambs (Nettleton and Entrican, 1995). These PI lambs are viraemic, antibody negative and constantly excrete virus. The virus spreads from sheep to sheep with PI animals being the most potent source of infection (Nettleton *et al.,* 1998). Several seroepidemiological studies were done

elsewhere as in the Austria, by means of an indirect ELISA that detected antibodies to Border disease virus (BDV) and bovine viral diarrhoea virus (BVDV) (Krametter-Frötscher *et al.*, 2007), in Quebec using the serum neutralization test (Lamontagne and Roy 1984), and in Egypt (Zaghawa, 1998). Serological surveys in many countries, have demonstrated widespread natural infection of pestiviruses in sheep (Nettleton, 1990) and goats (Loken, 1995). In Saudi Arabia, the situation of the pestiviruses infection is unknown, although clinical disease was observed in sheep and goats without laboratory confirmation. Therefore the aim of the present work was planned to clear up the sero-epidemiological situation of BVD infection among sheep and goats in Al-Ahsa region, Saudi Arabia.

MATERIALS and METHODS

Sampling:

A serological survey on sheep and goats were carried out and a total of 1471 serum samples obtained from 86 flocks were used in this study. The study area was Al-ahsa governorate of the Eastern Provence of Saudi Arabia. The collected blood samples were obtained from the Jugular vein using sterile syringes and vacutainer tubes. They were allowed to clot at ambient temperature and sequentially centrifuged at 4000g for 15 minutes, and the serum was separated and stored at –20 ºC until used.

ELISA test for detection of border disease virus antibodies:

A commercial indirect ELISA kits is used from SVANOVIR company, Sweden, article number 10-8100-02. The procedure was followed according to the producer.

Interpretation of the results:

1- The optical density (OD) values in wells coated with BDV are corrected by subtracting the OD values of the corresponding wells containing the control antigen (OD corr.). All control and samples OD values should be corrected before results are interpretated.
2- To ensure the validity of the positive control serum should have an OD corr. Greater than 1.0 and the negative control should have an OD less than 0.15.
3- Calculation of cut-off (A) which equals the double of OD corrected negative control, if it is more than 0.25 it is used as cut-off and if it is less than 0.25 so the cut-off is 0.25.

4- All samples with an OD greater than the cut-off, are considered as positive, while all samples with an OD lower than the cut-off are considered as negative.

RESULTS

Table 1: Distribution of border disease seropositive sheep and goats according to age.

		Age group			Total
		≤ 2years	3 – 5 years	> 5 years	
Sheep	Number tested	110.00	339.00	99.00	548.00
	Number Seropositive	18.00	83.00	29.00	130.00
	% Seropositive	16.36	24.48	29.29	23.72
Goats	Number tested	166.00	546.00	211.00	923.00
	Number seropositive	16.00	77.00	28.00	121.00
	% Seropositive	9.64	14.10	13.27	13.11
Sheep and goats	Number Tested	276.00	885.00	310.00	1471.00
	Number Seropositive	34.00	160.00	57.00	251.00
	% Seropositive	12.32	18.08	18.39	17.06

Non signifecant year effect within sheep CHI = 5.1 P= .078
Non signifecant year effect within goats CHI = 2.23 P= .327
Non signifecant year effect within sheep & GOAT CHI = 5.42 P= .0665
Signifecant effect between sheep and goats CHI=27.368 P=0.01

Table 2: Distribution of border disease seropositive sheep and goats according to breed.

	Breed	Number Tested	Number Positive	% Positive
Sheep	Najdi	244	48	19.67
	Awassi	238	72	30.25
	Sawakni	24	4	16.67
	Barbari	38	5	13.16
	Other	4	1	25.00
		548	130	23.72
Goats	Ardi	570	69	12.11
	Shami	283	38	13.43
	Jabali	67	14	20.90
	Other	3	0	0
		923	121	13.11

SIGNEFICANT CHI VALUE BETWEEN SHEEP BREED = 10.83 P=.0285
NO SIGNEFICANT CHI FOR BETWEEN GOAT BREEDS = 4.55 P= .20

Table 3: Sox susceptibilty of sheep and goat to BVD infection.

		SEX		Total
		Female	Male	
Sheep	Number tested	491.00	57.00	548.00
	Number Seropositive	117.00	13.00	130.00
	% Seropositive	23.83	22.81	23.72
Goats	Number tested	833.00	90.00	923.00
	Number seropositive	106.00	15.00	121.00
	% Seropositive	12.73	16.67	13.11

NO SEX EFFECT CHI VALUE = .03 P=.86 FOR SHEEP ALONE
= 1.11 P= .29 GOAT ALONE
= .36 P= .54 FOR SHEEP & GOAT COMPARISONE

DISCUSSION

During the period of 2006-2007 the serological examination of the tested samples (Table 1) elucidate that 23.72% and 13.11% of the examined sheep (n=548) and goats (n=923) were serologically positive to BDV infection, respectively. Such results may refer to the existence and the persistence of BDV among sheep and goats population at Al-Ahsa region of Kingdom Saudi Arabia.

The prevalence of antibodies to pestiviruses in four federal states of Austria was carried out on 4931 sheep, in 377 flocks, by indirect ELISA to detected antibodies to Border disease virus and bovine viral diarrhoea virus. The mean individual prevalence of BD virus antibodies was 29.4 % (Krametter-Frötscher *et al.*, 2007 and Schiefer *et al.*, 2006). This result is nearly in accordance with the results of the present work. Another seroepidemiological study of border disease in various areas of Quebec was conducted and revealed that 10.9% and 16% of sheep and goats, respectively, gave a positive reaction (Lamontagne and Roy, 1984). These results are slightly lower than that obtained in the present work. Such difference may attributed to the test used in that investigation is the serum neutralization test to BVD virus and in the present work we used an specific ELISA to Border disease virus. ELISA is more sensitive than virus neutralization test (Nettleton, 1990).

The distribution of border disease seropositive sheep and goats according to age is illustrated in Table (1). Sheep were classified according to age into 3 groups, (110) $\leq$ 2 years, (339) 3 – 5 years and

(99) > 5 years from which 18 (16.36 %), 83 (24.48 %) and 29 (29.29 %) were positive respectively. From the same table goats were classified according to age into 3 groups, (166) ≤ 2 years, (546) 3 – 5 years and (211) > 5 years from which 16 (9.64 %), 77 (14.10 %) and 28 (13.37 %) were positive respectively. It is clear that there is significant effect of age on the seroprevalence border disease virus antibodies, such results are in accordance with that obtained by (Lamontagne and Roy 1984).

Table (2) showed the distribution of border disease seropositive sheep and goats according to breed. Regarding to sheep breeds, 244 (Najdi), 238 (Awassi), 24 (Swakni), 38 (Barbari) and 4 others were tested from which 48 (19.67 %), 72 (30.25 %), 4 (16.67 %), 5 (13.16 %) and 1 (25.00 %) were positive respectively. In respect to goat breeds 570 (Ardi), 283 (Shami), 67 (Jabali) and 3 (other) were tested from which 69 (12.11 %), 38 (13.43 %), 14 (20.90 %) and 0 (0.00 %) were positive respectively.

The effect of sex on border disease seropositive sheep and goats is shown in Table (3). 491 female and 57 male sheep were tested from which 117 (23.83 %) and 13 (22.81%) were positive. In the same site 833 female and 90 male goat were tested from which 106 (12.73 %) and 15 (16.67 %) were positive.

The offered data on Table (1) indicate that there is a correlation between the age of the tested animals and seroprevalence of BDV infection. The data in Tables (1 & 2) revealed that there is a highly significant increase (PC 0.01) in the susceptibility rate of sheep to BVD infection than goats (X^2= 27.369). Breeds of sheep varies significantly in the seroprevalence of border disease virus antibodies as the Awassi is the higher and the barbari is the lowerest. Statistical analysis showed non significant effect of sex on BDV susceptibility, such results are in accordance to Lamontagne and Roy (1984) and Nettleton (1990). There is also no influence of communal alpine pasturing on the spread of pestiviruses among sheep and goats in Austria (Krametter-Froetscher *et al.,* 2007).

It can be concluded that BDV infection in sheep and goats is serologically established in Al-Ahsa region in Saudi Arabia and further studies are needed for clinical observation of the disease and laboratory detection of the etiologic agent by isolation procedure or by molecular techniques, to draw the map of the border disease and or bovine viral diarrhae.

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