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Bovine Leukosis in Holstein Cattle in Saudi Arabia As Evidenced By Presence of Specific Antibodies

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In the present study, serum samples obtained from Holstein cattle, native cattle, sheep, goats and camels, in Al-Qassim and surrounding regions, were tested for *Bovine Leukaemia Virus* (BLV) antibodies. For different animal species, agar gel immunodiffusion was carried out using commercial diagnostic reagents. All samples of herds having positive cases with immunodiffusion were tested with ELISA. Positive cases were recorded among samples of the Holstein cattle with both serological methods. Prevalence rates differed from one farm to another and with both serological tests. With agar gel immunodiffusion, the prevalence rates in 3 herds were 43.13%, 21.76% and 6.25%. The prevalence rates with ELISA in the same herds were 52.94%, 34.01% and 6.25%, respectively. Tested samples of two more herds showed no evidence of infection. Concerning native cattle and other animal species tested, no positive case was recorded. The results indicated that bovine leukosis exists among dairy farms especially the ones owning Holstein animals that have been imported from the United States of America. This finding should be taken seriously as the infection is insidious and one case in a farm can spread the infection easily to the rest of the animals. Therefore, farms should be tested for the infection and positive animals are to be discarded under a national control program.

INTRODUCTION

Bovine leukaemia (Syn. Enzootic bovine leukosis [EBL] is a malignant neoplasia of the lymphoreticular system. The disease attracted the attention early in the 19th century in several European countries, such as Denmark and Germany, where clusters of herds with a high incidence of disease suggested a viral aetiology (Darcel, 1996).

Bovine leukaemia virus (BLV) is transmitted horizontally within herds under conditions of close contact. Transmission occurs primarily by the transfer of blood and/or lymphocytes between animals. This can be via trauma, restraint devices, gloves used for rectal examination, reuse of needles and surgical instruments, and rarely by insects does acting as mechanical vector (Donovan, 1999 and Coffin, 1992).

The geographic distribution of BLV is worldwide and the reservoir is the infected cattle. Approximately, 25% of the dairy cattle in the United States are infected with BLV, leading to large economic losses due to death of cattle, decreased milk yield, condemnation of slaughter, and veterinary expenses (Kettmann *et al.*, 1994).

Bovine Leukaemia Virus has been shown to be infectious for several animal species other than cattle, including sheep and goats. Under natural conditions, the oncogenic potential of BLV appears to be expressed only in cattle and sheep. The ovine isolates are regarded as bovine leukaemia. Although BLV replicates in human cell tissue cultures, humans are not known to be infected. However, some human

serum samples gave positive results when tested serologically for BLV antibodies (Donovan, 1999 and Murphy *et al.*, 1999).

In Saudi Arabia, many private dairy projects have been established and most of which preferred the American Holstein dairy cows. In a FAO report, it was mentioned that BLV infection was detected among dairy cows in Saudi Arabia. However, no article was published to confirm this record and the type of affected cattle breeds was not mentioned (FAO, 1994).

Therefore, this study was conducted to clear out the situation in Saudi Arabia represented by Al-Qassim and neighboring regions. Beside cattle, serum samples of sheep, goats and camels were also tested.

MATERIALS AND METHODS

Serum samples

Cattle Serum samples were collected from Holstein dairy cows of different farms in Al-Qassim and neighboring regions. Meanwhile, samples were randomly collected from native cows, sheep, goats and camels in small flocks in Al-Qassim region. Each serum was separated from jugular vein blood drawn from animals into a plain vacutainer tube.

Diagnostic materials

***Bovine Leukosis AGID Kit*:**

This kit was purchased from SYNBIOTICS EUROPE (69367 LYON Cedex 07, France). The kit consists of freeze-dried BLV antigen, BLV freeze-dried positive control reference serum and diluents for the antigen and the serum.

Bovine Leukaemia Virus Antibody Test Kit:

This is an ELISA kit for serum testing (screening and verification). It is supplied by IDEXX Laboratories (One IDEXX Drive, Westbrook, Maine 04092, USA). The kit consists of BLV antigen coated plates, BLV antigen/normal host cells (BLV/NHC) coated strips, Anti-bovine IgG-horseradish peroxidase conjugate, anti-BLV positive control serum, BLV-negative control serum, 3', 3', 5', 5', tetramethylbenzidine (TMB) substrate, dilution and washing buffers, as well as stop solution.

Agar gel immunodiffusion test (AGID):

It was performed according to the manufacturer instructions. Agar gel (0.8%) was prepared in Tris buffer (0.2 M and pH 7.2) with 8.5% NaCl (OIE, 1996). After agar solidification, wells were punched in a regular manner using a rubber borer and guiding mold to give a central well with 6 peripheral wells. Serum samples were loaded in the peripheral wells in alternation with the positive control while the BLV antigen was loaded into the central well. Plates were incubated in a humid chamber. After 48-72 hours, positive results were indicated by precipitation lines that merge with the precipitation lines of the positive control serum.

Enzyme linked immunosorbent assay (ELISA):

For confirmation, all samples of herds having positive results with AGID test were tested by ELISA following the protocol described by the kit supplying laboratories. Samples were diluted 1/25 in the dilution buffer followed by loading into the BLV antigen coated wells. After incubation and washing, wells were charged with the diluted anti-bovine conjugate followed by a

second incubation and washing steps. In parallel, undiluted positive and negative control BLV sera were loaded into certain wells in dublicates and treated similarly. The TMB substrate was distributed into the test wells and left for 15 minutes followed by stopping the reaction. The reading of the absorbance was at 650 nm for samples and controls using an ELISA reader (STAT FAX® 3200, Awareness Technology Inc., Palm City, FL., USA). Interpretation of results was carried out based on the OD values of samples as well as positive (PC) and negative (NC) controls. Positive samples were reconfirmed by a verification ELISA using the BLV/NHC coated strips to exclude the background. The verification steps were as above except that each sample was loaded into two wells, one coated with BLV antigen and the other coated with normal host cell antigen.

Interpretation of the ELISA results

The S/P ratio of each sample was calculated as follows:

$$S/P = \frac{\text{Sample OD} - \text{negative control OD}}{\text{Positive control OD} - \text{negative control OD}}$$

Samples with S/P $\geq$ 0.5 were considered positive and reconfirmed by verification. In verification, samples with S/CNH $\geq$ 1.8 were considered positive for BLV antibodies

$$S/CNH = \frac{\text{OD BLV screening positive}}{\text{OD NHC}}$$

OD: absorbance at 650 nm wavelength

RESULTS AND DISCUSSION

Bovine Leukaemia Virus belongs to the *Oncoviruses* of the family *Retroviridae* (Greek *onkos* meaning tumor) because of its potential to produce neoplasia (Coffin, 1992; 1996 and Coffin *et al.*, 1997).

Beside cattle, BLV has been reported to be infectious for several animal species including sheep and goats. Under natural conditions, the oncogenic potential of BLV appears to be expressed only in cattle and sheep. Although BLV replicates in human cell tissue cultures and BLV antibodies were detected in some human cases, humans are not known to be infected (Donovan, 1999; Murphy *et al.*, 1999 and Van der Marten *et al.*, 1990).

Infection, once established, appears to be lifelong and no treatment or control other than removal of seropositive animals immediately. Test and removal programs have been adapted by several European countries, such as Germany and Denmark. These countries require that imported cattle be serologically negative for BLV infection using standardized tests (Kettmann *et al.*, 1994 and Murphy *et al.*, 1999).

A vague notice has been reported by the FAO office about detection of BLV infection among dairy cows in Saudi Arabia (FAO, 1994).

In the present study, the Holstein breed of cattle was started with as it is preferred for its high milk yield and most of the farms in Saudi Arabia have their cows imported from the United States of America. It has been stated that about 25% of the dairy cattle in the United States are infected with BLV (Kettmann *et al.*, 1994).

For the survey, two serological assays were carried out and both of them are prescribed by the OIE of Europe. These tests are

agar AGID and ELISA tests. Agar gel immunodiffusion (AGID) test is the official test used by the American USDA to test animals for export. In contrast, ELISA is the first prescribed test in the European countries (OIE, 1996).

One of the advantages of the AGID test is its suitability for different animal species and therefore serum samples of sheep, goat and camels were tested in parallel with the bovine samples. On the other hand, the available ELISA kit is bovine specific and it was, therefore, utilized as a confirmatory test for bovine AGIDT positive herds.

Representative bovine samples (n=266) were randomly collected from Holstein cows belonging to 5 different private dairy herds in Al-Qassim and surrounding regions in the central part of Saudi Arabia. The results obtained with the AGID test indicate existence of BLV infection in 3 of the tested herds. As shown in table (1), herd prevalence ranged from 0-43.13% as tested samples of two herds showed no positive cases while samples of the positive 3 farms showed prevalence rate of 6.25%, 43.13% and 21.76%. This is the real picture of bovine leukosis which is known to be a herd disease due to its transmission through animal trauma, restraint devices, gloves used for rectal examination, reuse of needles and surgical instruments (Donovan, 1999). Moreover, 3-20% of the BLV transmission has been reported to be through milk, colostrums and *in utero* (Murphy *et al*., 1999). Nevertheless, the infection can be transmitted from an affected herd to an unaffected one through trading (animal sailing) which maximizes the problem.

Samples of sheep (n=60), goats (n=50), native cattle (45) and camels (n=45) were tested with the BLV-AGID test. Fortunately, no one case was positive which can be attributed to their being away from the foreign breed dairy farms. A previous study reported similar results with camels (Wernery and Werenery, 1990). At this situation, it is worthy to suspect that the dairy farms having Holstein cows represent the most important focuses of suspected BLV infection.

Positive AGIDT samples were reconfirmed with ELISA where AGID-positive samples were also positive with ELISA. To evaluate both serological tests, all samples of herds having positive AGIDT cases were tested with ELISA. Unfortunately, more samples reacted positively with ELISA and the individual herd as well as the general prevalence rates increased (Table 2 and figure 1). From table 2, one can see the big difference in the prevalence rates within and between herds. The obtained results are not surprising as ELISA is being known for its high sensitivity if compared to the AGID test. ELISA as the first is a primary test while the AGID is a secondary one. From table (3), it is noticed that the S/P ratio of most positive samples was at high range of positivity (1.1-2 or more). On the other hand, most of the negative samples showed S/P ratio less than and far away from the border line value ($\geq$0.5). Consequently, no doubtful results were obtained with ELISA while some samples gave very weak results with AGID test. As a result, we suggest AGID to be used as a screening test and the positive cases are to be discarded while the rest of animals in the positive herds should be tested with ELISA. This is because AGID test is easy to perform and is not that costly and its specificity and sensitivity are good enough to be relied on for eradication

of positive cases. As AGID test could not detect weak positive or early infected cases, it should be repeated at 2-3 month intervals during which the test will be able to detect positive cases that escaped the preceding survey. This is the case when immunodiffusion and ELISA are used to serosurvey animals for a certain disease (Chikamatsu *et al.*, 1989 and Garg and Chandiramani, 1985). When testing other animal species, no doubt that AGID test is the test of choice.

BLV infection, once established, appears to be lifelong and no treatment or control other than removal of seropositive animals immediately. Test and removal programs have been adapted by several European countries, such as Germany and Denmark. These countries require that imported cattle be serologically negative for BLV infection using standardized tests (Donovan, 1999; Kettmann *et al.*, 1994 and Schwartz and Levy, 1994).

In Canada and the United States, individual owners have undertaken test and removal programs on a voluntary basis. Unfortunately, in both countries, national programs are not in action yet which maximizes the danger when importing cattle from there (Murphy *et al.*, 1999).

The situation is not that bad yet in Saudi Arabia and BLV infection is almost localized and not widespread. Under such kind of situation, the only way of fighting the infection is serological testing of susceptible animals and removal of positive ones immediately. This, undoubtedly needs a governmental-controlled programs based on conclusive and decisive rules and legislations.

From the available literature, testing is better repeated at 2- to 3-month intervals and the length of time needed for obtaining a BLV-free herd varies according to the initial prevalence of infection. In most herds, one year is almost enough to accomplish the target. In case of high prevalence, it is recommended to segregate seropositive and seronegative animals to facilitate the task. Calves from infected dams should be isolated, tested, and only allowed to join the herd if they are seronegative at 6 months of age (Murphy *et al.*, 1999 and Van der Marten and Mille, 1990).

Table (I): Prevalence rate of bovine leukosis in different Holstein dairy cattle herds as detected by the agar gel immunodiffusion test (AGIDT)

Herd No.	No. of tested samples	No. of positive samples	Positivity percentage
1	32	2	6.25%
2	51	22	43.13%
3	20	0	0.00%
4	16	0	0.00%
5	147	32	21.76%
Total	266	56	21.05%

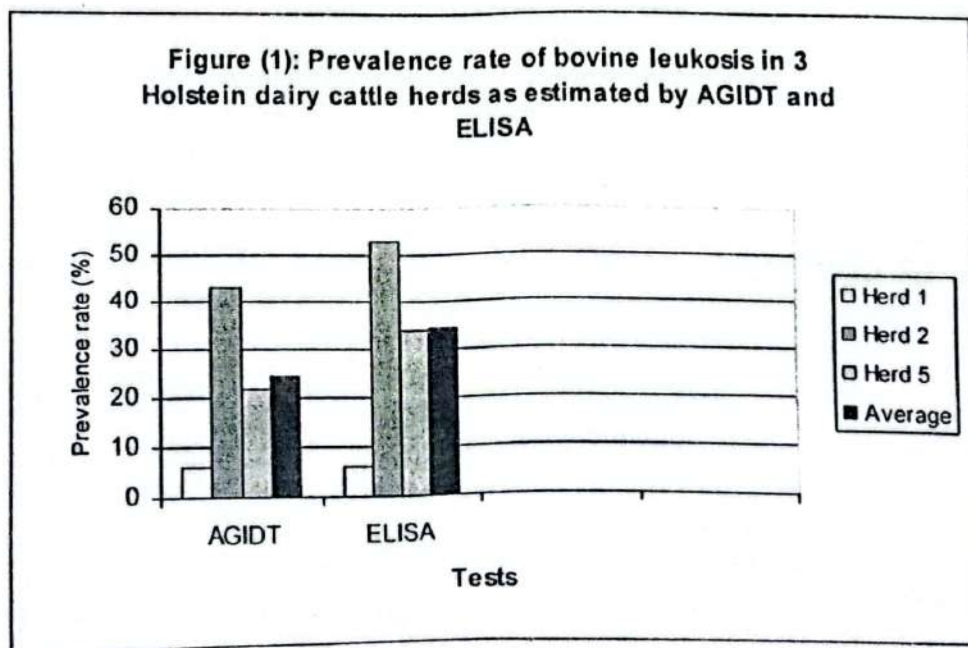
Table (II): Prevalence rate of BLV infection in Holstein dairy cattle by AGID test and ELISA

Herd No.	No. of tested samples	AGIDT-positive samples		ELISA-positive samples	
		No.	%	No.	%
1	32	2	6.25%	2	6.25%
2	51	22	43.13%	27	52.94%
5	147	32	21.76%	50	34.01%
Total	230	56	24.34%	79	34.34%

Table (III): S/P ratio of ELISA-tested cow serum samples for BLV infection

S/P ratio*	<0.1	0.1-0.19	0.2-0.49	0.5- 1.0	1.1-2.0 or more
Sample No.	41	39	10	8	132
Interpretation	negative	negative	negative	positive	positive

*S/P ratio was estimated according to the kit supplier directions from the O.D. values of positive and negative controls as well as test samples



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