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RESEARCH ARTICLE

ENZOOTIC BOVINE LEUKOSIS IN TUNISIA ASSESSMENT OF THE SITUATION IN SEVEN FARMS IN THE ORGANIZED SECTOR

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ABSTRACT

Enzootic bovine leukemia is a disease of cattle in which the epidemiological cause of the disease is bovine leukosis virus (BLV). Cattle with LSA almost invariably die either suddenly or weeks or months after the onset of clinical signs. The BLV infection has been associated with direct production losses (milk loss, premature voluntary culling and reduced slaughter value, mortality loss and abortion, reproductive loss) and treatment costs (veterinary services, medication cost and extra farm labour cost), with reduced export competitiveness and increased consumer concerns regarding safety. In Tunisia, cattle breeding represents an important component in agricultural production, in the national economy, in the social field and in human health. It has been classified among the priority sectors because it generates two strategic products (milk and meat). This work consists of a descriptive sero-epidemiological survey to estimate the rate of leucosis infection of herds and animals belonging to seven farms in the organized sector of three Tunisian governorates: Jandouba, Kasserine and Sidi Bouzid located in the north and central west. A total random sample of 393 dairy cows over five years of age was surveyed on the seven farms. The ELISA test is the test used in our epidemiological investigation; it made it possible to obtain the following results; animal's infection rate level is $21.37 \pm 1.44\%$. The results found in this survey, show that leucosis infection is gaining ground compared to those found in previous surveys, hence the need to apply adequate hygienic measures and to establish an action plan in order to reduce an important risk of amplification.

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INTRODUCTION

Enzootic bovine leukemia is a disease of cattle in which the epidemiological cause of the disease is bovine leukosis virus (BLV), a deltaretrovirus in the Retroviridae family, but other factors contribute to development of the condition (Nuotio L). L'infection leucosique peut survenir à n'importe quel âge et elle est généralement subclinique, those with LSA have symptoms which reflect the site of the tumor (Maresca *et al.* 2015). Enzootic bovine leukosis characterized either by persistent lymphocytosis (PL) or lymphosarcoma formation (LSA), or both (Nuotio L, 2003). Cattle with LSA almost invariably die either suddenly or weeks or months after the onset of clinical signs. There is a genetic predisposition to both PL and LSA which occur independently but often coincidentally. Iatrogenic infection through blood-contaminated needles and surgical instruments is probably the major route of transmission in most settings.

Field transmission of BLV requires direct prolonged contact between animals or an exchange of blood, exudates, or tissues. Spread of BLV between herds can occur via infected animals. Vertical transmission, semen, or hematophagous insects probably account for only a small proportion of cases (Juleriana *et al.* 2016). The BLV infection has been associated with direct production losses (milk loss, premature voluntary culling and reduced slaughter value, mortality loss and abortion, reproductive loss) and treatment costs (veterinary services, medication cost and extra farm labour cost), with reduced export competitiveness and increased consumer concerns regarding safety. There is persistent immunodeficiency and increased susceptibility to other diseases (Ott *et al.* 2003, Acaite J, 2007). Annual Economic Losses to the U.S. Dairy Industry Related to Leukosis cattle are estimated at \$285 million for breeders. However, the subclinical impact of infection by bovine leukosis virus on cow longevity is not included in these estimates.

EBL has a worldwide distribution and prevalence varies between countries. In some countries, EBL has been successfully uprooted via national control and elimination programs. The disease has nearly been eradicated from the European Union, Australia and New Zealand (Hemmatzadeh F., 2015). Other countries present a contrasting epidemiological situation with a large prevalence of EBL, such as the USA [Bartelett *et al.*, 2014, Ott *et al.*, 2003, La Dronka *et al.*, 2018), Argentina (Gutierrez *et al.* 2012), Canada (Nekouei *et al.*, 2015), and Japan (Murakami *et al.*, 2011, Murakami *et al.*, 2013). In Tunisia, cattle breeding represents an important component in agricultural production, in the national economy, in the social field and in human health.

It has been classified among the priority sectors because it generates two strategic products (milk and meat). In 2017, the Tunisian livestock included 437,000 female units, producing 1424 million liters. Cattle numbers are distributed differently over the different regions of the country with 72% of the herd in the north, 24% in the center and only 4% in the south. Dairy cows are raised according to four breeding systems: the intensive integrated and organized system, the semi-intensive non-integrated system, the "off-farm" system and the traditional or extensive system. It should be noted that the organized sector (state and semi-state farms) has 86% of the purebred herd and accounts for approximately 82% of total milk production. (site givlait, tunisieindustrie.nat.tn, Rejeb Gharbi F, 2007, Darej, 2017). This work consists of a descriptive sero-epidemiological survey to estimate the rate of leucosis infection of herds and animals belonging to seven farms in the organized sector of three Tunisian governorates: Jandouba, Kasserine and Sidi Bouzid located in the north and central west.

MATERIALS AND METHODS

This survey was carried out in seven farms of the organized sector belonging to three Tunisian governorates, namely a governorate in the northwest: Jandouba (Region 1) with 5 farms, a governorate in the central-west: Kasserine (Region 2) with a single farm and a governorate in the center: Sidi Bouzid (Region 3) with a single farm (Map 1). These farms were selected on the basis of the stability of the dairy production activity and the availability of data on the operation. A total random sample of 393 dairy cows over five years of age was surveyed on the seven farms as summarized in Table 1.

The cows tested were mainly Holsteins. The blood samples taken from the cows were transferred to the laboratory of the Department of Contagious Diseases, Zoonoses and Sanitary Legislation of the National School of Veterinary Medicine of Sidi Thabet, Tunisia either directly or after centrifugation and recovery of the serum to carry out the indirect ELISA test for the detection of antibodies directed against the Enzootic Bovine Leukosis virus. The ELISA kit used for this investigation is the ELISA kit for the detection of antibodies directed against the Enzootic Bovine Leukosis Virus (BLV), IDEXX Leukosis Serum Screening, France. Statistical analysis Data were analyzed with SPSS (Version 23.0. Armonk, NY: IBM Corp.). Univariate analysis is through Pearson's chi-square test was performed to assess the strength of the association between the farms and the tests' results at 5% significance threshold value.



Carte 1 . Location of the surveyed farms: (1) Jandouba; (2) Kasserine and (3) Sidi Bouzid

RÉSULTATS

In the three regions studied (Tab 2), region 1 with an infection rate of $34.70 \pm 6.28\%$ has the highest infection rate at the animal level. In region 2, this rate is estimated at $3 \pm 3.33\%$ and in region 3 at $5.5 \pm 5\%$. The infection rate obtained at the animal level is $21.37 \pm 1.44\%$. As shown in Table 3, only one farm had no seropositive animals among the seven studied. The other six farms contained at least one animal with positive serology. Bovine leukemia is primarily an adult disease (Gregory *et al.* 2017). For this work, only animals older than 5 years were investigated. For the study of the results obtained according to age, the values obtained were divided into two groups; a first group of the animals whose age is less than seven years and a second group whose age is greater than or equal to seven years. For animals under seven years of age, only animals in region 1 gave positive serologies. For animals older than 7 years, only farms 2 and 3 contained animals with positive serology. This result is in agreement with those of Sevik (Sevik, Avci, and Ince 2015) in Turkey and Khudhair (Khudhair *et al.* 2016) in Iraq.

DISCUSSION

The ELISA test is the test used in our epidemiological investigation since it is more sensitive than AGID, however, both tests have high sensitivity and specificity and OMSA has introduced them as the standard tests for sero-epidemiological studies (Mousavi *et al.* . 2014). The infection rate obtained at the animal level is $21.37 \pm 1.44\%$.

Table 1. Total number of cows and number of cows tested per farm

Gouvernorate	Region 1				Region 2		Region 3	Total
Farm	N°1	N°2	N°3	N°4	N°5	N°6	N°7	
Total effective	50	490	377	1232	149	405	390	3093
Number of cows tested	15	66	57	23	60	100	72	393

Table 2. Animal infection rate by region

Regions	Number of cows tested	Number of cows tested with positive result	Infection rate (%)	Confidence Interval (%)
Region 1	221	77	34.70	±6.28
Region 2	100	3	3	±3.33
Region 3	72	4	5.5	±5

Table 3. Number of animals with positive serology among the animals tested per farm

	Number of cows tested	Number of cows tested with positive result	Infection rate (%)
Farm 1	15	3	20
Farm 2	66	36	54.54
Farm 3	57	34	59.64
Farm 4	23	4	17.39
Farm 5	60	0	0
Farm 6	100	3	3
Farm 7	72	4	5.55

Table 3. Number of positive animals by age

	Age less than 7 years old			Age greater than or equal to 7		
	Tested animals	Positive animals	Infection rate (%)	Tested animals	Positive animals	Infection rate (%)
Region 1	111	20	18.01±7	110	57	51.81±9
Region 2	45	0	0	55	3	5.45±5.97
Region 3	34	0	0	38	4	10.52

Table 4. Number of seropositive animals tested under 7 years of age

	Number of cows tested	Number of cows tested with positive result	Infection rate (%)
Farm 1	15	3	20
Farm 2	28	13	46.42
Farm 3	3	1	30
Farm 4	19	3	15.78
Farm 5	46	0	0
Farm 6	45	0	0
Farm 7	34	0	0

This rate is close to those found in China, Japan, the Philippines and Iran which are respectively 21.09%, 18.6%, 23.01% and 25.4%. (Murakami *et al.*, 2011, Mousavi *et al.*, 2014). However, BLV infection is still common in North American dairy herds, averaging ~90% herd prevalence and ~40% within-herd prevalence (Kuczewski A *et al.*, 2020). The number of cattle on farms 2-3-4-6 and 7 exceeds 300 head. Farms 2-3 and 4 have the highest infection rate. Other studies report a higher prevalence of BLV in herds of more than 250 cattle. Intensive milk production in these dairy herds is based on a free-stall system which leads to increased physical contact between cattle and could be one of the main causes of BLV transmission (Yuji *et al.*, 1983). In a study, Kobayashi *et al.* showed that a free-stall system was a risk factor for BLV transmission (Kobayashi *et al.*, 2010). Bovine leukemia is primarily an adult disease (Gregory *et al.*, 2017). In its presentation of the diseases, OMSA, speaks of an infection at any age in cattle, including the embryonic stage. Most infections are subclinical but a certain proportion of cattle over 3 years old (~30%) will develop persistent lymphocytosis, and a smaller proportion will develop lymphosarcoma (tumors) in various internal organs (OMSA).

The presence of infected animals in the population aged less than seven years in our study has been noted in other studies. This variation in the age of infected animals could be due to different routes of transmission or different types of management practices that allow greater contact between animals of different ages as animals enter reproductive age and are exposed to older cattle, many of which have high viral load (Monti *et al.*, 2007, John *et al.*, 2020).

CONCLUSION

The disease is of significant economic importance as it causes losses due to reduced milk production, fertility, and the degradation of the market value of cattle from infected farms (Kuczewski A. *et al.*, 2022). Generally, likely routes of BLV transmission are animal-to-animal contact, blood contact during natural breeding, vertical (e.g., in utero) transmission, periparturient transmission, and transmission through milk, colostrum, or both. Additionally, some herd characteristics and management strategies with potential roles in BLV transmission were identified (Kuczewski A. *et al.*, 2022). The results found in this survey, show that leucosal infection is

gaining ground compared to those found in previous surveys, hence the need to apply adequate hygienic measures and to establish an action plan in order to reduce an important risk of amplification. The application of good hygiene practices associated with implementation of a prophylaxis program specific to bovine leukosis have enable its eradication in several countries such as France (Rautureau et Perin,2012, EFSA, 2015)

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