

Epidemiological survey on virus diseases of cattle in North West Syria.

M. Giangaspero*, G. Vacirca*, E. Vanopdenbosch**, H. Blondeel**

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Summary

Dairy cows from 19 farms from different locations in North West Syria were tested for BVD, BRSV, PI3, IBR, BHV4, BPS and adeno viruses (IIF and AGID methods). BRSV and BHV4 were the prevalent infections with 88% and 83.2% of seropositivity, respectively. PI3 and BVD infections were also frequent, showing 62% and 58%, respectively. IBR, adeno and BPS showed a lower prevalence (31%, 26% and 11%).

Résumé

Les vaches laitières de 19 élevages provenant de différentes localisations du nord ouest de la Syrie ont été testées pour les virus BVD, BRSV, PI3, BHV4, BPS et adeno (méthodes IIF et AGID). BRSV et BHV4 étaient les infections prévalentes avec respectivement 88% et 83,2% de séropositivité. Les infections PI3 et BVD étaient également fréquentes avec 62% et 58% de positivité. IBR, adeno et BPS montraient une prévalence plus basse (31%, 26% et 11%).

Introduction

Dairy farming system is an emerging sector of the Syrian Zoo-technics, especially in the North west and coast provinces. Cattle consists mainly of imported breeds, about 450,000 Holstein Friesians, which represent the second important domestic animals in the country, after small ruminants (Ministry of Agriculture and Agrarian Reform, Department of Statistics, 1991, unpublished data). Health control still remains an aspect to be developed. This includes the study of the epidemiology of the various pathogens which may occur in cattle; only scarce information are available on Syria, especially concerning viruses (Sellers R.F. and Taylor W.P. Investigations on virus diseases of ruminants in Syria, 1978-1981. Animal Virus Research Institute, Pirbright, UK., unpublished data; 9). In addition, one of the major constraints to productivity are the reproductive disorders, causing heavy losses. In some farms, serious difficulties are reported concerning the fertility of cows, for example high rate of artificial insemination (A.I.) failure; in others, the abortion rate reaches up to the 60%. Studies on Brucellosis showed a very low distribution of this disease in Syria (Central Veterinary Laboratory, Damascus, unpublished data).

Considering the frequency and the importance of some viral diseases in Europe and the European origin of the Syrian herds, and that the same clinical respiratory and reproductive disorders are observed in both cattle populations an examination of the viral origin of these troubles was justified. In Europe, respiratory disorders are mainly attributed to Bovine Respiratory Syncytial Virus (BRSV), Infectious Bovine Rhinotracheitis (IBR), Parainfluenza virus type 3 (PI3) and Bovine Viral Diarrhea (BVD).

In order to obtain a clear and actual picture of the epidemiology of these viral infections in the country, a serological survey was undertaken to determine the prevalence in North west Syrian provinces with relatively large number of cattle. Adenovirus and Bovine Papular Stomatitis (BPS) were also

screened. Bovine Herpesvirus 4 (BHV4) was considered as one of the possible agents involved in the etiopathology of the genital disorders affecting the Syrian herds. Pressure of BHV4 infection and its clinical signs were verified comparing serological results with individual anamnestic data, taking into account that seropositive animals are always virus carriers, a common characteristic of Herpesvirus infections (12).

The PI3 virus, Myxoviridae family, is a common respiratory pathogen of cattle, occurring mainly in association with other viral and bacterial infections. This virus is involved in the etiopathology of Enzootic calf pneumonia and Shipping fever, with high morbidity, and mortality may reach 10-20% (4,14). Clinical signs can vary from mild forms with only coughing, to severe pneumonia with systemic (pyrexia, anorexia, depression) and respiratory (tachypnea, hyperpnea, dyspnea) signs. Lesions also may vary from mild rhinitis and tracheobronchitis to extensive pneumonia (2).

Bovine Herpesvirus 1 is the causal agent of IBR and other pathologies such as Infectious Pustular Vulvovaginitis Infectious Balanoposthitis, abortions and encephalitis in calves (5). IBR is characterized by inflammation of the upper respiratory tract with, initially serous then mucopurulent, nasal and ocular discharge, pyrexia, anorexia and sudden reduction of milk production. Abortions, during the third trimester of pregnancy, are frequent in the course of the disease. Infections were reported in many countries showing high morbidity (from 20 to 100%) and 1-10% mortality (14).

The Bovine Respiratory Syncytial Virus (BRSV), a pneumovirus, is one of the most diffuse pathogens affecting the respiratory tract in cattle (10). In typical outbreaks, suddenly 80-90% of a certain group may show symptoms. Clinical signs start with coughing, nasal discharge, conjunctivitis and fever. Thereafter, some animals develop bronchopneumonia (type A) or emphysema with alveolar cells destruction

* Institute of Special Pathology and Veterinary Medical Clinic, Faculty of Veterinary Medicine, University of Milan. Via Celoria 10. 20133 Milan, Italy

** National Institute for Veterinary Research (NIVR). Groeselenberg 99, 1080 Brussels, Belgium

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(type B). Type A is found in dairy calves of a few week of age while type B lesions are encountered in emphysematous lungs of older beef calves. Lesions are often complicated by bacterial infections. Mortality may be relevant.

Bovine Viral Diarrhea is a cosmopolitan infectious disease caused by pestivirus. Its main characteristic is an important immunodepressive action. Vertical transmission may occur causing immunotolerance. Symptoms are not limited to the gastrointestinal tract, reproductive and respiratory disorders occur frequently with high losses.

Bovine adenoviruses are widespread pathogens, causing fatal outbreaks of pneumoenteritis in calves with severe losses, especially in large herds (1). The disease may start with respiratory symptoms (serous nasal and ocular discharge, coughing) followed by diarrhea. Fever and anorexia complete the clinical signs. Secondary bacterial infections can aggravate the disease. Reduction of weight gain due to malabsorption is a common sequel of the infection (3).

Bovine Herpesvirus type 4 (BHV4) is a pathogen related to various disorders in cattle including metritis and abortion (7,8,13).

Bovine Papular Stomatitis (BPS) is a worldwide distributed disease in cattle, caused by a Parapoxvirus, usually a mild, afebrile, clinic form with spontaneous regression. BPS is characterized by proliferative lesions which may be confused with other exanthematic diseases, particularly the early changes of Foot and Mouth disease (FMD) (6).

Material and Methods

Sample collection

Blood samples were collected from 243 Holstein Friesian cows originating from 19 farms in different locations of the North west Syrian provinces (Aleppo, Hama and Homs). The survey encompassed cattle raised both commercially (3 dairy state farms with about 1,000 cows each) and traditionally. The sera were stored at -20°C until examination.

The reproductive performance of some animals from the intensive farms was seriously affected and the anamnesis showed A.I. failure (repeated more than 5 times), retention of placenta and abortions (up to 60% in one of the farms during the last 3 years). In a family managed farm, consisting of only 20 lactating cows, the health status and reproduction were apparently good and no abortion were recorded during the previous years.

Indirect Immuno-Fluorescence Test (IIF)

The antibody detection by IIF was performed following the drop method as described by Wellemans and Leunen (11). The antigen substrate for the different viruses was obtained as followed: for BRSV, RB 94 strain grown on primary foetal bovine kidney cells; for IBR, BHV4, PI3 and BPS viruses, Belgian field strains grown on primary foetal bovine testicle cells (National Institute for Veterinary Research, NIVR, Brussels). The sera were tested following a base 3 dilution starting from 1:15, in phosphate-buffered saline (PBS). Hyperimmunised calf sera were used as positive control (NIVR). The second antibody for bovine serum consisted of anti-bovine IgG, obtained from hyperimmunised goat (GAB), conjugated with fluorescein-isothiocyanate; the conjugate was tested for non-specific binding before and after lyophilization (NIVR). The

conjugate was used at a dilution of 1:250, in PBS with 1:20,000 W/V Evans blue. The slides were observed under an epi-fluorescence microscope (Nikon X2F-EFD2). Titres $\geq 1:15$ were considered positive, excluding non-specific reactions.

Agar Gel Immuno Diffusion Test (AGID)

Adenovirus type 3, grown on primary foetal bovine testicle cells and concentrated by ultracentrifugation (NIVR), was used for the execution of the test. Hyperimmunised calf serum against adenovirus type 3 was used as the positive control. Petri plates containing 15 ml of agar gel with 6 circular peripheral and a central 5 mm diameter wells were used. The distance between the central well and the peripheral wells was 5 mm. Seventy μl of antigen suspension were placed in the central well; the same quantity of control serum and test sera were placed in the peripheral wells. The plates were incubated in a humid chamber at room temperature and read at 48-72 hours. Curved lines and lines of identity with the control serum lines were considered positive, excluding non-specific reactions.

Results

Using IIF and AGID tests, seropositivity to the 7 viral antigens screened was found in animals from all the areas studied (Table 1). Except for BHV4, titres of antibodies were generally low, not exceeding 1:1215 (Table 2). Intensive farms were more affected by viral infections showing a higher frequency of seropositivity for BRSV, PI3, IBR, BHV4 and BPS than farms with low number of animals. Only BVD and adenovirus infections were higher in the traditional farms (Table 3). Mixed infections were encountered relatively frequently. Most common combinations of viral infections are reported in Table 4. Occurrence of other mixed infections did not exceed 5%. Single infections were less frequent, only BRSV reached a level of 8.4%.

TABLE 1

Percentage of cattle positive for antibodies against BVD, PI3, BRSV, IBR, BHV4, BPS (IIF method) and Adeno (AGID method) viruses in North west Syria.

Antigen	n samples	% positive
BRSV	183	88
BHV4	167	83.2
PI3	198	62.1
BVD	174	58
IBR	243	31.3
Adeno	167	26.3
BPS	72	11.1

TABLE 2

Range of antibody titres (IIF method) against BVD, PI3, BRSV and BPS viruses, found in cattle sera in North west Syria. Values expressed in percentage.

IIF Titre	BVD	PI3	BRSV	IBR	BPS	BHV4
Negative	42	37.9	12	68.7	88.9	16.8
1 15	36.8	21.7	14.8	14.8	4.2	3.6
1 45	13.8	23.7	33.3	11.5	6.9	0.6
1 135	6.3	9.6	21.3	4.1	—	4.2
1 405	0.6	4.5	15.8	0.8	—	13.8
1 1,215	0.6	2.5	2.7	—	—	19.8
1 3,645	—	—	—	—	—	28.7
1.10.935	—	—	—	—	—	9.5
1:32.805	—	—	—	—	—	2.4
1:98,415	—	—	—	—	—	0.6

TABLE 3

Comparison of seropositeness to virus infections in cattle from intensive or traditional farms. Values expressed in percentage.

Type of farm	Antigen						
	BVD	PI3	BRSV	IBR	BPS	Adeno	BHV4
Intensive	55.8	72.1	96.6	47.6	40	23.8	93.2
Traditional	70.4	33.3	52.8	6.2	6.4	45	10

TABLE 4

Identification of the most common mixed infections in cattle in North west Syria. Values expressed in percentage.

PI3 + BRSV	25.1
PI3 + BRSV + IBR	22.8
PI3 + BRSV + Adeno	15.6
BRSV + IBR	12.6

Two groups were identified: cows showing reproductive disorders ($n=55$) and healthy cows ($n=63$). Seropositivity to BHV4 was higher in the former group (91%) than in the latter one (68%). In addition, high titres of antibodies were more related to disorders of reproduction. In the first group, 54.5% of the samples showed titres $\geq 1:3,645$; in the healthy cows group, only the 19% showed such titres.

Discussion

The study has evidenced that the same viruses exist both in European and in Syrian cattle populations, showing epidemiological similarities with the same levels of seropositeness and a prevalence of BRSV, both in single and mixed infec-

tions, at farm level and in individual cases.

Infections were found both in the intensive dairy farms and in traditional ones, except for IBR which was either absent or rare in the latter.

Antibodies against IBR, PI3 and BVD were very low. Nevertheless, this indicates regular contact of animals with the antigens. The circulation of the viruses in these herds insures protection, but farms where most of the animals were IBR-seronegative are exposed to the risk of outbreaks of the diseases.

The occurrence of antibodies against BPS in Syrian cattle might suggest clinical cases which should be taken into account for the differential diagnosis of FMD according to the relevant importance of this disease.

The findings on BHV4 indicate the occurrence of the infection in the area studied, and its high prevalence particularly in the intensive dairy farming system. BHV4 possibly plays a role in the etiopathology of reproductive disorders in Syria and this may correspond to previous studies carried out in Europe and Africa (7,8,13). Further investigations will be needed to clarify the epidemiology of this infection and determine the full extent of the impact of BHV4 on cattle productivity in the country.

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Samenvatting: Melkkoeien uit 19 bedrijven, verspreid over verschillende streken van het noordwesten van Syrië, werden onderzocht op aanwezigheid van antistoffen tegenover BVD, BRSV, PI3, IBR, BHV4, BPS en adenovirus met indirecte immunofluorescentie en immunodiffusie in gelose. Voor BRSV en BHV4 werden respectievelijk 88% en 83,2% van de dieren seropositief bevonden. Voor PI3 en BVD waren respectievelijk 62% en 58% van de koeien seropositief. Voor andere virussen zoals IBR, adeno en BPS werden minder dieren positief bevonden, respectievelijk 31%, 26% en 11%.

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