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ПРИКЛАД МОДЕЛІ ОЦІНКИ РІВНЯ БІОЛОГІЧНОГО РИЗИКУ НА ФЕРМАХ НА ОСНОВІ ВИМОГ GAP (НАЛЕЖНОЇ СІЛЬСЬКОГОСПОДАРСЬКОЇ ПРАКТИКИ)

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Підвищення обізнаності щодо важливості відповідності вимогам щодо безпечного виробництва тваринницької продукції та забезпечення якості продукції (м'ясо, молоко, яйця) вимагає попереднього виконання певних програм, при яких може бути визначений рівень біологічної безпеки на фермах. Метою даної роботи є розробка моделі у вигляді веб-додатку, який дозволяє швидко оцінити рівень біологічної безпеки на фермах, визначити рівень відповідності необхідним стандартам, а також можливість використання різних методів для поліпшення робочих процесів.

EPIDEMIOLOGY OF BLUETONGUE IN FRANCE AND DEVELOPMENT OF MOLECULAR TOOLS FOR BTV AND EHDV TYPING IN THE FRENCH MARTINIQUE AND LA RÉUNION ISLANDS

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Bluetongue virus (BTV) and Epizootic haemorrhagic disease virus of deer (EHDV) are two species of the genus Orbivirus within the Reoviridae familly. Bluetongue virus (BTV) is the cause of bluetongue (BT), an insect-transmitted disease of domestic and wild ruminants [1, 2]. BTV infection occurs throughout much of the temperate and tropical regions of the world, coincident with the distribution of specific species of Culicoides biting midges that act as biological vectors of the virus [3, 4]. BT typically occurs when susceptible animal species are introduced into areas where virulent strains of BTV circulate, or when virulent strains of BTV extend their range into previously unexposed populations of ruminants. Following the recent northern European epidemic, BTV has spread far beyond the historical northern limits of its range [5].

BTV is the prototype member of the genus Orbivirus, family Reoviridae [6]. As a reovirus it has a segmented genome of double-stranded RNA (dsRNA) and a characteristic virion morphology and structure. On the basis of serotype-specific virus neutralization assays 24 distinct serotypes of BTV have been described to date, but a virus isolated from goats in Switzerland (Toggenburg virus) likely is a 25th serotype [7].

EHDV is morphologically, structurally and biologically similar to BTV; 7 serotypes of EHDV have been identified. EHDV is also transmitted by biting midges. EHDV which is present in the US but also in the North of Africa (Morocco, Algeria, Tunisia) is a real threat for the European livetosck.

From 1998 to 2006, five different BTV serotypes (1, 2, 4, 9 and 16) have spread throughout extensive portions of Mediterranean Europe [8, 9]. In 2006, BTV serotype 8 emerged unexpectedly in the North of Europe involving Belgium, France, Germany, Luxembourg and the Netherlands [5, 10]. In 2007, BTV-8 spread rapidly and widely throughout much of Europe, as to a lesser extent did BTV-1. In 2008, two other BTV serotypes were detected in Northern Europe: BTV-6 in the Netherlands and BTV-11 in Belgium [11, 12].

The European incursion of BTV has had a considerable negative economic impact, partly due to direct losses from mortality and reduced production in affected livestock but, more importantly, from the ban of ruminant trade between BTV-infected and non-infected areas.

To limit direct losses and in an effort to minimize the circulation of BTV and allow safe movements of animals, authorities from affected European countries undertook vaccination of livestock according to their individual national policies, the geographic distribution of the incurring BTV serotype(s), and the availability of appropriate vaccines [10, 13, 14]. Before 2005 only modified live virus vaccines were used in these national BTV vaccination campaigns and, except for Italy where all susceptible domestic ruminant species were vaccinated, only sheep were vaccinated [9, 10, 13]. After 2005, when inactivated vaccines became available, cattle, and goats were also vaccinated.

In 2011, the high vaccination coverage has permitted to control BT in Europe. No virus has been isolated in 2011 in the North of Europe (Belgium, the Netherlands, Germany) and in France.

This paper describes the epidemiological of BTV in France since 2006 and the development of BTV and EHDV type–specific RT-PCR which have allowed to characterize new strains isolated from the Martinique (in the Caribbean Sea) and the La Rйunion Island (in the Indian Ocean).

Moreover, BTV and EHDV are present in the Caribbean and in the Indian Ocean in particular in the French Islands (Martinique, Guadeloupe and la Rйunion).

BT EPIDEMIOLOGY IN FRANCE (mainland)

BTV serotype 8 was introduced in France in 2006. In 2007, the virus spread dramatically with more than 14 000 cases [5]. In 2008, more than 38 000 cases were reported. RT-PCR was developed to detect BTV genome [5]. BTV serotype 1 virus was also isolated in the South West of France. Molecular tools for typing were developed in our laboratory to detect BTV-1 and BTV-8. Vaccination with an inactivated vaccine was performed. All cattle, sheep and goats had to be vaccinated. In 2010, only one case was reported in the south of France and no case in 2011.

BT EPIDEMIOLOGY OF ORBIVUSES IN THE FRENCH ISLANDS

New molecular methods were developed in our laboratory to detect BT and EHD viruses in the French Islands (Martinique and la Rйunion Islands).

Materials and methods. Study No 1: Martinique

In 2006, 30 cows (viro and seronegative for BTV) exported from France to the Martinique Island were sampled 10, 20 and 30 days post-importation.

The sera were tested by a competition ELISA (BT C-ELISA IDvet) and EDTA blood samples were used for PCR and viral isolation on embryonated eggs.

A sequence analysis of segments 2 (this segment 2 encodes VP2 which is the outer capsid protein involved in serotype specific serological reactivity) of the 24 serotypes of BTV (DNAStar Megalign) allowed to select a consensus pair of primers.

This pair of primers was used in PCR to amplify a part of segments 2 of isolated strains. The amplification products obtained with these primers were sequenced and compared with those availabable on GenBank (BLASTN).

Study No 2: La Rйunion Island

In 2009, BTV-like clinical signs were reported in cattle of the French la Rйunion Island.

121 animals with clinical signs were sampled. EDTA blood samples were used for BTV, EHDV-group-specific RT-PCR and viral isolation on embryonated eggs.

The consensus pair of primers (as described above for BTV amplification) was used on BTV positive samples.

The segments 2 of the EHDV genome (encoding VP2) are clustered in 4 groups (A, B, C and D). We selected primers specific for each group. These pairs of primers were used in PCR to amplify a part of segments 2 of EHDV strains. The

amplification products obtained with these primers were sequenced and compared

(BLASTN) with those available on GenBank.

Results. Study No 1: Martinique

Thirty days post-importation, 56 % of animals seroconverted and 80 % were positive with a BTV group real-time RT-PCR.

The amplification products obtained with consensus primers from the RNA extract from isolated strains were sequenced and allowed to type the serotypes: 2, 9, 10, 17, 18, 22 and 24.

Sequence analysis of these strains showed that the origin of these viruses is unknown and that the serotypes 2, 10 and 17 do not originate from America.

Study N°2: La Rйunion Island

By BTV and EHDV-group-specific RT-PCR, 120 animals were detected as EHDV RT-PCR positive and 5 animals BTV and EHDV RT-PCR positive; moreover, one strain of BTV and 7 strains of EHDV were isolated in embryonated chicken eggs. The BTV virus was type as serotype 2.

The sequence of the amplification products (with the pair of primers specific of Group C) allowed to conclude that the EHDV serotype was 6. Sequence data showed that these strain was the cause of the EHD outbreak in the Island in 2003. This serotype 6 is also present in North Africa since 2006.

Discussion. BT vaccines may be used for different purposes or strategies, depending on the epidemiological situation of the affected area and policy desired. The main purposes of BT vaccination strategies are: (i) to prevent clinical disease, (ii) to limit the regional extension of BTV infection through reduction of the spread of the virus, (iii) to allow regional or country eradication of the disease based on the reduction of virus circulation, and (iv) to authorize the safe movement of susceptible animals between affected and free zones [14]. These are the goals that have driven European authorities to implement BTV vaccination campaigns since the incursion of BTV into Europe.

Since the initial introduction of BTV serotype 8 into Northern Europe in 2006, more than 100 million animals have been vaccinated and the incidence of BT disease has rapidly decreased as a result. Although herd immunity following natural infection as well as climatic conditions such cold winters that adversely impact vector insect populations have undoubtedly contributed, it is likely that the vaccination of livestock has had a major role in reducing virus circulation and even eradicating the virus from some areas of Europe.

Moreover, the development of molecular diagnosis methods for BTV and EHDV has been an important part of BTV monitoring in Europe. It has also greatly benefited from the availability of genomic sequences in international databases (GenBank,EMBL,...). The selection of primers for PCR and their validation requires access to the nucleotide sequences of viruses of different serotypes and many strains of the

same serotype. Since the emergence of the BTV serotype 8 in Europe, the laboratories have understood the need to share such genetic information for the benefit of the structures involved in the development of modern methods of diagnosis.

It is of importance to develop validated molecular typing methods for BTV and EHDV which are distributed worldwide and constitute an important threat to the European livestock.

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ЕПІДЕМІОЛОГІЯ БЛЮТАНГУ У ФРАНЦІЇ ТА РОЗРОБКА МОЛЕКУЛЯРНИХ МЕТОДІВ ДЛЯ ТИПУВАННЯ ВІРУСУ БЛЮТАНГУ І ВІРУСУ ЕПІЗООТИЧНОЇ ГЕМОРАГІЧНОЇ ХВОРОБИ В МАРТІНІЦІ ТА НА ОСТРОВАХ РЕЮНЬОН

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У статті описується епідеміологічна ситуація щодо вірусу блютангу у Франції з 2006 року і розробки видоспецифічної ПЛР реального часу для вірусу блютангу і вірусу епізоотичної геморагічної хвороби, що дозволить охарактеризувати нові штами, виділені в Мартініці (в Карибському морі) та на островах Ла Реюньон (в Індійському океані).