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EPIDEMIOLOGICAL SURVEY ON THE DIFFUSION OF THE FIRST SUBTYPE OF RABBIT HAEMORRHAGIC DISEASE VIRUS (RHDVa) IN SOME ITALIAN REGIONS

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ABSTRACT

In order to compare the rate of diffusion in the field of Rabbit Haemorrhagic Disease virus (RHDV) and its first subtype called RHDVa, we carried a study on the outbreaks of this disease occurred on 1997, 1998 and 1999 in different Italian regions. In addition, to rapidly distinguish between case of RHDV and RHDVa and to enlarge our capacity to detect new possible variants, we developed new MABs towards the RHDVa. A total of 334 cases of RHD were diagnosed, 47 of which resulted RHDVa. The data indicate that RHDVa was already present in most part of Italy with the exception of the Sicily Island. The high percentage (27%) was found in Lazio and Toscana. The data also showed that RHDVa is spreading quite rapidly on the field. The importance of such variant is discussed with reference to vaccine preparation and application.

INTRODUCTION

Rabbit haemorrhagic disease virus (RHDV) is a non-cultivable calicivirus that infects rabbits (*Oryctolagus cuniculus*) and causes outbreaks of an acute fatal hepatitis (OHLINGER 1990; CAPUCCI, 1991). The virus emerged in 1984 in China, coming out in Europe two years later where caused a succession of devastating epidemics both in commercial and wild rabbit populations. Due to the large extension and variety of rabbit populations, RHDV is practically impossible to eradicate and the correct use of the vaccine, together with good farm conduction, are the only tools available to keep under control its diffusion and protect rabbit breeding. At present RHDV is endemic in most parts of Europe and Asia and in some African countries. In Oceanic continent RHDV has been accidentally introduced in 1995 during experimental trials conducted to test its possible use as biological control of wild rabbit population considered as pest both in Australia and New Zealand.

Such as all other RNA viruses, RHDV is endowed of a considerable genetic variability that should be the base for a high antigenic variability, theoretically favoured by its large and rapid diffusion. In spite of this, RHDV exists as unique serotype and all the molecular epidemiological studies, carried out sequencing the gene of the RHDV capsid protein (VP60) of different isolates, did not detected consistent antigenic variation (percent of amino acid substitutions among VP60s less than 4%) up to the end of 1996 (LE GALL 1998; NOWOTNY 1997). Recently, two almost contemporary but independent studies, showed the presence in Italy and Germany of RHDV isolates with an antigenic profile clearly and consistently different from the original one (CAPUCCI 1998; SCHIRRMIEIER 1999). In the case of the Italian isolates, they did not reacted in ELISA test with the monoclonal antibody (MAB) 1H8, able to completely protect from the disease rabbits experimentally infected with RHDV (CAPUCCI

1995) and they were also less reactive with rabbit sera produced with the original RHDV isolate. Genetic analysis pointed out that the amino acids substitutions responsible for the antigenic change were mainly grouped between positions 344 and 370 of the VP60, segment that is included inside the main antigenic region of RHDV (CAPUCCI 1998). Importantly, vaccinated rabbits challenge with the RHDV variant did not show any sign of disease. Very similar data were also reported for the RHDV variant isolated in Germany, even if rabbits vaccinated with a lower dose showed to be less resistant to the variant than to the original strain of RHDV used as vaccine (SCHIRRMIEIER 1999). On account of the net genetic and antigenic difference found, the RHDV variant can be considered as a subtype of the original virus and we named it RHDVa.

In order to compare the rate of diffusion of RHDV and RHDVa in the field, we carried a study on the RHDV cases diagnosed in different Italian regions during 1997, 1998 and 1999. In addition, to rapidly distinguish between case of RHDV and RHDVa and to enlarge our capacity to detect new possible variants, we developed new MAbs towards the RHDVa.

MATERIAL AND METHODS

The different laboratories from the regions involved in the study sent to us the RHDV positive samples in order to mark them as RHDV or RHDVa. These samples (liver and spleen homogenates) were examined using the routine laboratory tests (sandwich ELISA, Western Blot, PCR) already described in details elsewhere (CAPUCCI, 1991, 1995,1998).

The ELISA test used for typing RHDV is based on the use of a panel of MAbs and it has been recently improved with the addition of antibodies produced towards the RHDVa subtype. The panel is constituted of three groups of MAbs: i) 1H8, 1H3 and 2A10 are specific for the original RHDV and bind to one of the main antigenic determinant on the RHDV capsid, probably constitute by two epitopes: the 1H8-1H3 epitope and 2A10 epitope. ii) MAbs 6F9, 3H6, 6H6, 1F10, 2B4 and 2G3 react both with RHDV and RHDVa and probably they bind to at least two different antigenic determinants, one of which is functionally important as the 1H8-1H3-2A10 epitope. iii) MAbs 3D4, 3B12, 2E1, 3D6 and 5D11 are specific for RHDVa and two of them, 5D11 and 3D6, recognise the same antigenic region recognise by 1H8-1H3-2A10 on RHDV. The MAb 5F5 is specific for European Brown Hare Syndrome virus (EBHSV) and it has been used as negative control.

Table 1: Summary of the number of RHDV and RHDVa subtype isolates

Year	Type of isolate	Friuli Veneto ²	Lombardia Emilia Romagna	Lazio Toscana	Sicilia	Total
1997	RHDV	20	68	42	61	191
	RHDVa	2	2	16	0	20
1998 ¹	RHDV	2	38	17	9	66
	RHDVa	4	3	7	0	14
1999	RHDV	n.d. ³	30	n.d.	n.d.	30
	RHDVa	n.d.	13	n.d.	n.d.	13
Total	RHDV	22	136	59	70	287
	RHDVa	6	18	23	0	47

¹ limited to the period January - August

² limited to the provinces of Udine, Gorizia and Treviso.

³ not done

RESULTS AND DISCUSSION

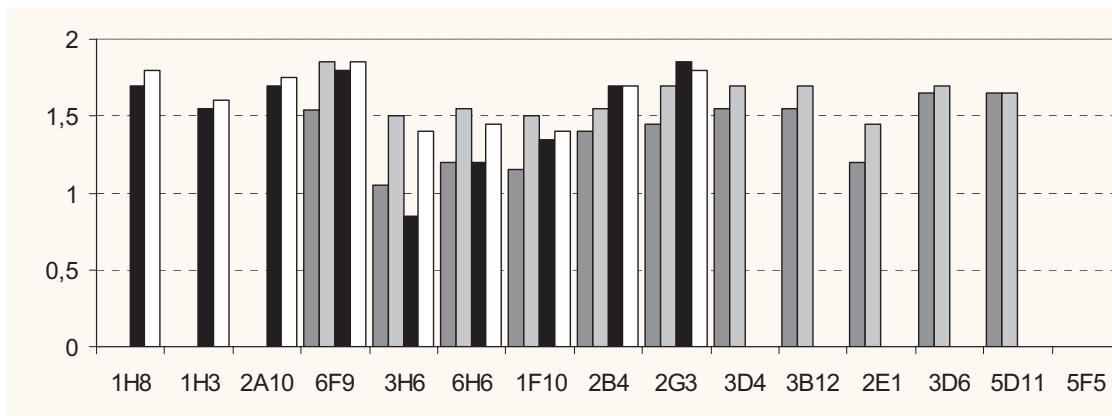
In table 1 we reported the results of the epidemiological survey. The data indicate that RHDVa was already present in most part of Italy already in 1997: the Sicily Island was the only region where all the isolates belonged to the original RHDV. Furthermore, the high percentage (27%) of RHDVa found in Lazio and Toscana suggests that probably the subtype occurred for the first time in Italy just in these regions. Indeed, one the first variants described was originating from Viterbo in the North of Lazio on 1997 (CAPUCCI 1998). The data also show that RHDVa is spreading quite rapidly on the field. In fact, while RHDVa was 3% of isolates in Lombardia - Emilia Romagna in 1997 it has been identified in 30% of the RHD cases in 1999, two years later.

From a more general point of view, these data seem to indicate a decrease of the incidence of RHD in the time. However, the number of outbreaks of the disease could be consistently underestimated, considering that most of the cases occur in little rural units, which owners have learnt to recognise the disease and, always more often, do not send samples to the diagnostic laboratory.

In Fig.1 is reported an example of the typing of RHDV using ELISA test. It is easy to see that the use of the MAbs panel, that includes high specific antibodies, allows a clear distinction between the subtype RHDVa (isolates n.343 and n.276) and the original RHDV (isolates n.540 and n.477). Furthermore, the panel should be able to detect most of the possible RHDV antigenic changes thank to the wide viral surface that it is able to probe.

To note that the partial sequence of the VP60 gene of some RHDVa confirmed the presence of most of the amino acid substitutions detected in the first two isolates (unpublished data), confirming the genetic specificity and stability of RHDVa.

Figure 1: ELISA analysis of RHDV isolated using an RHDV and RHDVa specific MAbs panel



Sandwich ELISA with a rabbit serum anti RHDV adsorbed onto the solid phase and IgG rabbit anti IgG mouse labelled with horseradish peroxidase (HRP) as final tracer. Grey bar: isolate 343; light grey bar: isolate 276; black bar: isolate 540; white bar isolate 477. Ordinates: absorbance units at 492 nm.

CONCLUSIONS

Our epidemiological survey demonstrates that the subtype RHDVa appeared in the regions of central Italy since 1997 and that from then on it diffused quite rapidly to other regions where it reached level comparable to the original RHDV. Considering the consistent antigenic difference between RHDV and RHDVa, it should be strongly recommended to continue to

follow the evolution of RHDV on the field, in order to be ready to use in the future the best antigen to prepare the vaccine.

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