Clinical evolution and diagnosis of an outbreak of European brown hare syndrome in hares reared in captivity *

M.L. ZANNI **, M.C. BENASSI **, M.T. SCICLUNA ***, A. LAVAZZA *** and L. CAPUCCI ***

Summary: The authors studied an outbreak of an acute form of European brown hare syndrome (EBHS) in captive hares. The farm involved had shown negative results in a previous serological test for EBHS conducted on approximately 8% of the animals. Hares which succumbed during the outbreak were submitted to an anatomo-pathological examination and the livers of these animals were collected for laboratory analysis. Examination by immunoelectron microscopy and enzyme-linked immunosorbent assay confirmed the diagnosis of EBHS virus (EBHSV). An initial serological survey conducted on the survivors twenty-two days after the outbreak demonstrated an immunological response against EBHSV. During the outbreak, data were collected on morbidity, mortality, incidence of the disease in various age groups, and also on the antigenic characteristics of the virus responsible for the outbreak.

KEYWORDS: Diagnosis – European brown hare syndrome – Hare – Hare farm – Lepus europaeus – Viral diseases.

INTRODUCTION

European brown hare syndrome (EBHS) – a typical disease of the species Lepus europaeus Pallas, 1778 – has been described for the last ten years in various northern European countries, in both wild and captive hares (5, 6, 8, 9, 10, 11, 12, 15, 17, 18). Recent studies have defined the viral aetiology of the disease (7, 13) and established the antigenic correlations between EBHS virus (EBHSV) and the virus of viral haemorrhagic disease of rabbits (VHD) (1, 3), a disease introduced only recently into Europe (15, 16, 17). However, VHD presents some epidemiological features which are different from those observed for EBHS, although many clinical-pathological similarities exist between the two diseases (3, 15).

This paper describes an acute outbreak of EBHS in hares reared in captivity. The aim of this study was to gather additional information on EBHS, especially on the evolution

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and clinical-epidemiological features of this disease, which is one of the most pressing health problems in hares at present. Further studies were also performed to define the relationship between EBHS and VHD.

MATERIALS AND METHODS

The farm on which the outbreak occurred was set up in 1983, in Castelvetro, Modena (Italy) by the Wildlife Centre of the Azieda per il riequilibrio faunistico ed ittico del territorio dell’Emilia Romagna (ARIS), in a fenced area of 3,600 m$^2$, which contained 82 cages placed in four rows, the rows being separated by a distance of 1.5 m. A small rabbitry was situated nearby, in an old building just outside the delimited area.

Previously, there had been two major disease outbreaks on the farm, each characterised by a sudden and high rate of mortality: the first incident occurred in November 1985, the second in October 1987 (Table I). On both occasions, it was impossible to identify the cause of the outbreak, but the sudden evolution and high mortality, as well as the symptomatology and pathological lesions observed, were suggestive of EBHS.

<table>
<thead>
<tr>
<th>TABLE I</th>
<th>Numbers of hares present on a farm in Castelvetro, Modena (Italy) and mortality rates recorded during two disease outbreaks in 1985 and 1987</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animals</td>
<td>18 November to 18 December 1985 (%)</td>
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<td>---------</td>
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</tr>
<tr>
<td>Total no. of animals</td>
<td>168</td>
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<tr>
<td>No. of adult animals</td>
<td>120</td>
</tr>
<tr>
<td>No. of young animals</td>
<td>48</td>
</tr>
<tr>
<td>Total mortality</td>
<td>67 (39.88)</td>
</tr>
<tr>
<td>Mortality in adult animals</td>
<td>40 (33.33)</td>
</tr>
<tr>
<td>Mortality in young animals</td>
<td>27 (56.25)</td>
</tr>
</tbody>
</table>

When the disease broke out in October 1990, there were 124 hares (62 adults and 62 young hares below the age of nine months) present on the farm (Fig. 1). The breeding stock (88 hares) was kept in the first and second rows, while the rest of the hares were kept in the fourth row (1-4 animals per cage). A serological investigation of ten animals (8.06% of the total) had been conducted, using an enzyme-linked immunosorbent assay (ELISA) (3, 14), 19 days previously, which partially established the EBHS-free status of the farm.

During the outbreak, necroscopic examinations were performed on all the dead animals. The organs were collected and 31 livers were homogenised and tested by ELISA (4), while 6 livers were also examined by immuno-electron microscopy (IEM) (3). The sandwich ELISA (4), using a rabbit anti-VHD virus (VHDV) hyperimmune
Different age groups of hares present on a farm in Castelvetro, Modena (Italy) immediately prior to an outbreak of the European brown hare syndrome in 1990

serum for capture, was performed with a variety of reagents as tracers (hare anti-EBHSV convalescent serum, rabbit anti-VHDV hyperimmune serum, VHDV-specific monoclonal antibodies [MAb] and VHDV MAb cross-reactive with EBHSV), the reactivity of which was shown to vary according to the antigen (EBHSV or VHDV) present in the diagnostic sample (2, 3).

A serological investigation was conducted on all survivors 22 and 87 days after the commencement of the outbreak. Sera were tested for anti-EBHSV and anti-VHDV antibodies by competition ELISAs specific for each type of antibody (3, 14). The titre of the serum sample corresponded to the dilution which resulted in 50% inhibition of the optical density (OD) value of the reaction given by the negative control serum.

RESULTS AND DISCUSSION

During the course of the outbreak, 73 hares (58.87%) died within 12 days, 42 were adults (67.74% of the total number of adults) and 31 (50%) of those born during the year.

Only mild symptoms were observed in both young and adult hares; a few hours prior to death, the animals stopped eating and showed depression. In some cases, excited nervous symptoms and epistaxis preceded death.

The mortality rate in the adults rapidly reached the peak value, whereas mortality was lower in the young hares, maintaining constant values for a longer period (Fig. 2).

Mortality was nil in animals below the age of 50 days, while mortality was low in hares over the age of 36 months (Fig. 3); the latter group was present during the 1987
Evolution of mortality observed in captive hares during an outbreak of the European brown hare syndrome on a farm in Castelvetro, Modena (Italy) in 1990.

Mortality in each age group of captive hares on a farm in Castelvetro, Modena (Italy) due to an outbreak of the European brown hare syndrome in 1990.
outbreak. Statistical analysis of the mortality relative to each age group (Table II) indicated a constant significant difference between the mortality in animals below the age of 50 days and mortality in other groups, except for the group aged 6-7 months and the group aged >36 months. A significant difference was also noted between the two groups of adult hares aged 9-36 months and >36 months.

**Table II**

*Comparison by the chi-square test of mortality due to the European brown hare syndrome observed in different age groups during an outbreak of the disease in hares reared in captivity on a farm in Castelvetro, Modena (Italy) in October 1990*

<table>
<thead>
<tr>
<th></th>
<th>&lt;50 days</th>
<th>50 days-3 months</th>
<th>3-4 months</th>
<th>6-7 months</th>
<th>7-8 months</th>
<th>8-9 months</th>
<th>9-36 months</th>
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<tbody>
<tr>
<td>50 days-3 months</td>
<td>*</td>
<td></td>
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<tr>
<td>3-4 months</td>
<td></td>
<td>***</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>6-7 months</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7-8 months</td>
<td>***</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8-9 months</td>
<td>***</td>
<td>*</td>
<td>NS</td>
<td>*</td>
<td>NS</td>
<td></td>
<td>***</td>
</tr>
<tr>
<td>9-36 months</td>
<td>***</td>
<td>***</td>
<td>NS</td>
<td>*</td>
<td>NS</td>
<td>NS</td>
<td>***</td>
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<tr>
<td>&gt;36 months</td>
<td>NS</td>
<td>NS</td>
<td>**</td>
<td>NS</td>
<td>*</td>
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</tr>
</tbody>
</table>

* * P<0.05  NS: not significant  ** P<0.01  *** P<0.005

No statistical relationship was found between susceptibility to EBHS and the sex of the animals, nor was there any relationship with consanguinity; in fact 58.06% of the surviving young hares were offspring of hares which died during the outbreak.

The necroscopic examination of carcasses revealed the classical lesions described for EBHS (6, 12, 13); these included degeneration and friability of the liver, splenomegaly, kidney congestion, uncoagulated blood in the thoracic and abdominal cavities, oedema and congestion of the trachea, and petechial disseminated haemorrhages of the mucosa and serosa.

The provisional diagnosis of EBHS, based on the clinical and epidemiological findings, was followed by laboratory identification of EBHSV in the homogenates of organs. The analysis carried out by IEM revealed the presence of viral particles which were morphologically identical to those usually present in cases of EBHS and indistinguishable from VHDV (as both have been proposed as new members of the family Caliciviridae) (15). The analysis of the homogenates by virological ELISA confirmed the presence of the viral agent in all samples and also the specific antigenic characteristics of the virus, which are always associated with cases of EBHS but never with cases of VHD. When EBHSV is present, the reactivity of the anti-EBHSV serum is significantly higher than that of the anti-VHDV serum; furthermore the cross-reactive VHDV MAb are positive, while the VHDV-specific MAb remain negative (2, 3).

No pathological signs were observed in the nearby rabbitry during the EBHS outbreak in the hare farm.
Sera collected 19 days before the outbreak gave negative results for anti-EBHSV antibodies, indicating susceptibility of animals on the farm to the disease; this was subsequently confirmed by the large number of deaths due to EBHSV. A large majority of the survivors gave positive results, with titres of ≤1/160 (75% of cases). The remainder had titres of ≥1/320, reaching a maximum of 1/2,560 (Fig. 4). In the second batch of sera, collected 87 days after the outbreak, overall titres were lower (Fig. 4). No statistical correlation was observed between antibody titre and the age of the animals.

Titres for VHDV were either negative (sera collected before the EBHS outbreak) or were between 8 and 128 times lower than those obtained for EBHSV (Fig. 4).

**FIG. 4**

Serological results of tests on sera collected 22 and 87 days after the commencement of an outbreak of the European brown hare syndrome in captive hares on a farm in Castelvetro, Modena (Italy) in 1990
CONCLUSIONS

Identification of the viral agent isolated during the outbreak confirmed the clinical-anatomopathological findings (which showed the better-known characteristics of EBHS) and concluded the diagnosis of an outbreak of EBHS on the farm.

Virological characterisation indicated the presence of a virus possessing an antigenic profile typical of EBHSV and different from that of VHDV. To date, EBHSV has been isolated exclusively in samples from hares, thus supporting the hypothesis that two distinct viral agents exist, each inducing a disease limited to a defined species (1, 3, 4).

Following identification of the disease involved in the outbreak as EBHS, the epidemiological value of the data collected during the outbreak was analysed. In regard to mortality, all animals younger than 50 days showed resistance to the disease but still seroconverted. In general, the mortality rate rose in accordance with the age of the animals, reaching a peak in those older than 9 months. The lower mortality rate (16.7%) recorded in animals older than 36 months could have been due to residual immunity due to a previous outbreak of EBHS (in 1987); however, this remains unconfirmed. The EBHS outbreak ended within 12 days of the first registered death, but peak mortality occurred within four days; the contagious nature and acute character of the clinical form of the disease can be deduced from the high percentage of deaths (60%) already registered by that time.

The serological investigation demonstrated that nearly all survivors had specific positive titres for EBHSV, probably due to seroconversion (partially confirmed by the negative titres of sera tested 19 days before the outbreak). The serological response of the survivors was not homogeneous; this could not be correlated to any particular condition related to the farm.

The titres for VHDV were constantly and significantly lower than those obtained for EBHSV. This is in agreement with the antigenic results mentioned above and with cross-serology results obtained previously (3, 14). Both of these factors indicate the existence of two serologically distinct viruses.

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Résumé : Les auteurs ont étudié la phase aiguë du syndrome du lièvre brun européen (European brown hare syndrome : EBHS) lors d'une épidémie survenue dans un élevage. L'épreuve sérologique pour la recherche de l'EBHS, préalablement pratiquée sur 8 % environ des animaux de l'exploitation concernée, a fourni des résultats négatifs. Les lièvres morts pendant l'épidémie ont été soumis à un examen anatomo-pathologique et leurs foies ont fait l'objet d'analyses en laboratoire. Le diagnostic du virus de l'EBHS a été confirmé à l'aide de la microscopie immuno-électronique et de l'épreuve enzyme-linked immunosorbent assay (ELISA). Une première enquête sérologique, pratiquée sur les animaux survivants 22 jours après l'apparition de la maladie, a révélé l'existence d'une réponse immunitaire contre le virus de l'EBHS. Des données
concernant la morbidité, la mortalité et l’incidence de la maladie dans des groupes d’âges différents ainsi que les caractères antigéniques du virus responsable ont été recueillies au cours de l’épidémie.


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Resumen: Los autores estudiaron la fase aguda de un brote del síndrome de la liebre parda europea (European brown hare syndrome: EBHS) que se declaró en un establecimiento de cría de liebres. La prueba serológica para investigar esta enfermedad, practicada previamente en aproximadamente el 8% de los animales, había dado resultados negativos. Las liebres muertas durante el brote fueron sometidas a un examen anatomo-patológico y se analizaron sus hígados en laboratorio. El diagnóstico del virus del EBHS se confirmó con ayuda de la microscopía inmunoelectrónica y de la prueba inmunoenzimática (ELISA). Una primera investigación serológica que se llevó a cabo en animales sobrevivientes 22 días después de declarada la enfermedad, mostró la existencia de una respuesta inmunitaria contra el virus del EBHS. Durante el brote pudieron recogerse datos sobre la morbilidad, la mortalidad y la incidencia de la enfermedad en grupos de liebres de edad diferente, así como sobre las características antigenicas del virus causal.


* * *

REFERENCES


