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The transmission of Jembrana disease, a lentivirus disease of *Bos javanicus* cattle

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SUMMARY

Methods of transmission of Jembrana disease, an acute and severe disease of Bali cattle (Bos javanicus) caused by a recently-identified bovine lentivirus known as Jembrana disease virus, are described. During the acute disease virus can be detected in saliva and milk. There is evidence of direct transmission from acutely affected animals in close contact with susceptible cattle, possibly by virus in these secretions infecting cattle by the conjunctival, intranasal or oral routes, by which it was possible to infect cattle experimentally. During the acute disease the titre of infectious virus in blood is high, about 10^8 50% cattle infectious units (ID_{50})/ml, and it is probable that the virus is also transmitted mechanically by haematophagous arthropods. Recovered cattle are also a potential but probably infrequent source of infection; recovered cattle are persistently viraemic but the titre of infectious virus in blood decreases to about 10^1 ID_{50}/ml by 60 days after recovery from the acute disease, and virus cannot be detected in secretions.

INTRODUCTION

Jembrana disease is an acute and severe disease of domesticated banteng or Bali cattle (*Bos javanicus* syn. *Bos sondaicus*) in Indonesia with a case fatality rate of about 20%. The incubation period is short (5–12 days) [1]. The principal lesions are an intense non-follicular proliferation of lymphoblastoid cells in lymphoid organs, and a lymphoid infiltrate in other visceral organs [2]. Infection of other bovine species induces only a mild or subclinical infection [3]. The disease is caused by Jembrana disease virus (JDV), a recently-described lentivirus that is antigenically and genetically related to, but distinguishable from, bovine immunodeficiency-like virus (BIV) [4, 5]. The acute nature of Jembrana disease with recovery of most affected animals and no recurrence of disease in the recovered animals [6] is unusual for a lentivirus.

The mechanism of transmission of Jembrana disease is poorly understood. When it first occurred on Bali island in 1964 it apparently required about 12 months to spread throughout the island [7]. The disease has since occurred in three

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other areas of Indonesia, Lampung and West Sumatra provinces in Sumatra island, and in East Java [8]. It is now endemic in all these areas but there has been limited spread of the disease from the endemic areas to adjacent areas.

This report describes epidemiological aspects of the disease, and studies designed to explain how transmission of Jembrana disease might occur, including the detection of virus in tissues and secretions of affected animals at intervals after infection and attempts to transmit the disease by various routes of inoculation.

MATERIALS AND METHODS

Jembrana disease virus

The Tabanan/87 strain of the Jembrana disease virus [6] was used in all experiments. The virus was maintained as previously described [9]. Briefly, spleen tissue from an experimentally infected Bali animal was frozen at −70 °C. When required, 1 ml of a 10% homogenate of the spleen, in Dulbecco's modified Eagle's medium (DMEM) with penicillin (100 units/ml) and streptomycin (100 μg/ml), was inoculated into susceptible Bali cattle. Heparinized blood was collected from the inoculated cattle on the second or third day of the febrile reaction, the plasma was immediately separated by centrifugation and used as a source of virus.

Bali cattle

All Bali cattle used for experimental infections were obtained from Nusa Penida, a small island adjacent to Bali, where Jembrana disease has not been detected, and antibody against JDV has not been detected [8]. These cattle were maintained in screened animal rooms and were all susceptible to experimental infection [1, 6].

Titration of virus

The titre of JDV in blood of four cattle at intervals after experimental infection was determined using methods similar to those previously described [6]. Briefly, the four animals were inoculated simultaneously with a 1/10 dilution of plasma suspension from an infected animal, prepared as described above. At 24 h intervals equal volumes of heparinized blood from each animal were pooled, 10-fold dilutions of the pooled blood were prepared, and 1 ml of each dilution was inoculated into two susceptible Bali cattle. The inoculated animals were monitored for clinical signs of Jembrana disease. A 50% infectious dose (ID_{50}) was determined as the highest dilution of the inoculum producing Jembrana disease in at least one of the two inoculated animals.

Detection of Jembrana disease virus in body secretions

Saliva, urine, milk, semen and faeces were collected from cattle at intervals after infection, and each sample separately inoculated into susceptible Bali cattle to detect infectious JDV. Saliva was collected from one infected animal by inserting a large cotton pledget into the lateral buccal cavity, expressed into a sterile container, diluted 1/5 in DMEM with 10% foetal bovine serum (FBS), clarified by centrifugation at 2100 g for 15 min, and 5 ml of the diluted saliva inoculated intravenously into each of two cattle. Urine was collected directly from the

urinary bladder of one animal immediately after it was killed, and from one animal at micturition, filtered through 220 nm membrane filters, and 5 ml of each sample inoculated intravenously into each of two cattle. Rectal faecal samples from two animals, infected on separate occasions, were diluted 1/5 (w/v) in DMEM with 10% FBS, centrifuged at 2100 g for 15 min, and 5 ml of the supernatant of each sample inoculated intraperitoneally into two cattle. Semen samples were collected with an artificial vagina from three young bulls trained for semen collection and infected on separate occasions. The samples were immediately diluted 1/5 with sterile PBS, pH 7·2 and 5 ml of each sample were inoculated intraperitoneally into each of two cattle. Milk was collected from a lactating cow, and 5 ml was inoculated intraperitoneally into each of two cattle.

Infection of cattle by oral, intranasal and conjunctival routes

A single batch of plasma from an infected animal, containing 10^8 ID_{50}/ml, was used to infect cattle by the oral, nasal and conjunctival routes. One ml of plasma was placed into the buccal cavity of two cattle, 0·2 ml into the nares of two cattle, and 0·2 ml into the conjunctival sac of two cattle. The oral and intranasal infections were repeated using a different plasma source. The infected cattle were observed for at least 14 days after infection for clinical signs of Jembrana disease. A clinical diagnosis of Jembrana disease was confirmed by necropsy of the animals and the detection of typical gross and histological lesions [2].

Serological tests

Serum samples were collected from cattle in a village in the Jembrana district, Bali, where Jembrana disease is endemic. Owners of the cattle were identified and the serum samples were examined for antibody to JDV by an enzyme-linked immunosorbent assay (ELISA) as previously described [10].

RESULTS

Titre of JDV in blood or plasma of cattle at intervals after infection

The approximate titre of JDV in blood of infected Bali cattle was determined at intervals after infection and the results are shown in Table 1. The titres (ID_{50}/ml) in blood were $< 10^4$ until 1 day before the onset of fever when they increased to $\geqslant 10^4$. Titres of 10^8 were detected on the second and third day of the febrile reaction, and 10^5 1 day after the end of the febrile period. The titre decreased to 10^2 32 days after infection and a low titre of about 10^1 was detected 47 and 72 days after infection.

Detection of virus in body secretions

The results of inoculation of body secretions from animals at various phases of Jembrana disease are shown in Table 2. Virus was detected in saliva, milk and urine during the febrile phase of the disease. All animals inoculated with the saliva and milk samples collected during this phase developed Jembrana disease, but only one of two animals inoculated with urine collected from the bladder 2 days after the start of the febrile phase developed Jembrana disease and no animals reacted which were inoculated with urine collected at micturition. Virus was not

Table 1. *The titre of infectious virus in peripheral blood of Bali cattle at intervals after experimental infection with JDV*

Days after infection*	1	2	3	4	5	7	8	12	32	47	72
Titre (log ID_{50}/ml)	< 4	< 4	< 4	> 4	$\geqslant 4$	8	8	5	2	1	1

* The febrile period in the sampled animals commenced 6 days after infection and persisted for 6 days.

Table 2. *Detection of JDV in secretions/excretions of cattle at intervals after infection*

		Phase of clinical signs in donor animal						
				Post-febrile phase (days after febrile phase)				
Sample	Volume	Pre-febrile phase	Febrile phase	1	7	13	27	90
Saliva	1 ml	NT*	2/2†	0/2	NT	NT	NT	NT
Urine	5 ml	NT	1/4	NT	0/2	NT	0/2	NT
Milk	5 ml	NT	2/2	NT	NT	0/2	NT	NT
Semen	1 ml	0/2‡	NT	NT	0/6	NT	0/2	0/2
Faeces	1 g	NT	0/4	NT	NT	NT	NT	NT

* NT, not tested.

† The virus was detected by inoculation of susceptible cattle with tissue from infected cattle and the development of clinical Jembrana disease in the inoculated cattle. Figures indicate number of inoculated cattle developing Jembrana disease/total number of cattle inoculated. Each individual sample was inoculated into two susceptible cattle.

‡ Sample collected 4 days post-infection and 3 days before the onset of the febrile reaction.

detected in saliva, milk and urine collected after the febrile phase of the disease. The virus was not detected in faecal samples collected during or after the febrile phase. It was not possible to collect semen from animals during the febrile phase of the disease and the virus was not detected in semen before the febrile phase or at any stage after the febrile period.

Experimental infection of cattle by oral, intranasal and conjunctival routes

After oral infection with Jembrana disease virus, one of two cattle developed Jembrana disease 6 days after infection. In a repeat experiment using a different plasma source, a similar result was obtained and one of two cattle developed Jembrana disease 8 days after infection.

After intranasal infection, one of two cattle developed Jembrana disease 9 days after infection. In a repeat experiment using a different plasma source, both inoculated cattle developed Jembrana disease, one animal 9 days after infection and one animal 13 days after infection. After conjunctival infection, both animals that were infected developed Jembrana disease 8 days after infection.

Serological studies

Serum samples were obtained from 77 cattle 1–4 years of age owned by 38 individual farmers located in a single village in the Jembrana district of Bali island where Jembrana disease is endemic. Thirty-seven of the farmers owned two cattle and one owned three cattle. The cattle owned by 13 farmers were all positive for

antibodies to JDV, the cattle owned by 18 farmers were all negative for antibodies to JDV, and cattle owned by 7 farmers gave mixed antibody-negative and antibody-positive results.

DISCUSSION

Jembrana disease is endemic in Bali and outbreaks of the disease with a low number of affected cattle in a small area are common. Based on an early hypothesis that the disease had a rickettsial aetiology [11] and known role of arthropods in the transmission of many rickettsial diseases, arthropods have been suspected to be involved in its transmission, and insecticides have been used to assist in control of disease outbreaks (unpublished observations). The role of arthropods in the disease has, however, never been confirmed and the disease has now been shown to be caused by a lentivirus [4, 9].

Several features of the disease and the virus are possibly relevant in the development of hypotheses on the method of transmission of the disease. First, during the acute clinical disease there was a high titre of infectious virus of about 10^8 ID_{50}/ml in blood of affected animals but in recovered animals the titre of virus declined to approximately 10 ID_{50}/ml. Second, virus was detected in secretions of infected animals only during the acute febrile phase of the disease. Third, infection of animals was possible not only by systemic inoculation of JDV into animals but also by infection of animals via the oral and intranasal routes and by instillation of JDV onto the conjunctival mucous membranes. Fourth, transmission of the disease to susceptible cattle from experimentally infected cattle has been observed when the cattle were kept in the same animal room [1]. Finally, there has been limited spread of the disease in Indonesia from endemic areas to adjacent areas [8].

There appear to be two distinct phases of virus replication and secretion of virus in affected animals: one phase during the acute febrile phase of the disease when there is a high titre of virus in blood and secretion of virus in saliva and in milk of lactating animals; a second phase following the acute disease when there is a low titred persistent viraemia and when it was not possible to detect virus in secretions.

The high titre of virus in blood of approximately 10^8 ID_{50}/ml during the acute clinical phase of Jembrana disease suggests that during this period there would be a high probability that the disease could be transmitted by the mechanical transfer of blood or blood-contaminated products from affected to susceptible cattle. In contrast, during and after the recovery period when the titre of virus declines to low levels of approximately 10 ID_{50}/ml, the chance of mechanical transmission by blood or blood-contaminated products would be relatively low.

Two methods of mechanical transmission of the virus in blood from cattle during the acute disease are probable: by multi-use needles during vaccination programmes, and by arthropods. The high titre of the virus in blood of 10^8 ID_{50}/ml during the acute disease suggests that vaccination procedures involving the multiple use of a single syringe and needle, sometimes practised in the areas where Jembrana disease is endemic, could mechanically transmit virus. This mode of transmission has been reported for equine infectious anaemia virus [13]. It is also possible that transmission of the virus on the mouth parts of haematophagous arthropods during interrupted feeding would be possible.

Transmission by haematophagous arthropods such as *Tabanus* species of equine infectious anaemia virus occurs from acutely infected horses, in which virus titres of about 10^6 infectious virus particles/ml of blood have been detected [12, 13], less than is detected during Jembrana disease. However, if mechanical transmission of JDV by arthropods does occur, this has not been responsible for extensive spread of Jembrana disease from endemic to adjacent areas: the disease has not spread from Bali island to two closely adjacent islands, Nusa Penida and Lombok, since the disease initially occurred in Bali in 1964, and there has been limited spread of the disease from the endemic area of Lampung province in Sumatra island to adjacent areas since the disease occurred there in 1976 [8].

In addition to possible transmission by mechanical transfer of JDV in blood there is evidence that during the acute phase of the disease it is contagious. Cross-infection from infected to susceptible cattle housed in direct contact with each other in screened animal rooms was previously reported [6]; the disease in the in-contact susceptible cattle occurred approximately 14 days after acute signs of the disease were detected in the inoculated animals. The detection of virus in saliva and milk, and possibly in urine during the febrile stage of the disease, and the ability to transmit the disease by oral, intranasal and conjunctival inoculation of the virus, suggests these routes of excretion and infection could be involved. However, as virus was only detected in secretions during the acute stage of the disease, when there is also a high level viraemia in affected cattle, transmission via these routes would be likely only from cattle with the acute disease and where there was intimate contact between affected and susceptible cattle.

Further evidence of the need for contact between animals for the transmission of Jembrana disease is that cattle owned by individual farmers tended to be either all infected (antibody-positive) or all uninfected (antibody-negative), suggesting that cross-infection of animals owned by the same farmer had occurred to a greater extent than between animals owned by different farmers in the same village. Cattle owned by individual farmers were frequently tethered together while grazing, and housed in isolation from other cattle; there is frequent intimate contact between these cattle but minimal direct contact between these cattle and other cattle in the same village. Even if the transfer of JDV between cattle owned by the same farmer was not the result of contact with infected body fluids, if an arthropod vector was involved it would suggest that arthropod transmission occurs to a greater extent if there are only short distances involved.

Transmission of JDV in semen during natural mating or by artificial insemination would not appear to be a likely means of dissemination of JDV, or if it does occur it is an infrequent event, as the virus could not be detected in semen before and immediately after the febrile phase of the disease.

Transmission of JDV in milk, demonstrated to be an important mode of transmission of the caprine arthritis-encephalitis lentivirus [14] is also a possible method of transmission of JDV. The virus was detected in milk and the disease could be transmitted by oral instillation of virus into susceptible cattle. However, the ability to detect JDV in milk only during the acute febrile phase of the disease suggests this route of infection is of limited importance.

Bali cattle are viraemic for at least 2 years after recovery from clinical disease [6] but the role of these persistently viraemic recovered animals in the transmission

of the disease is unknown. The titre of virus in blood 72 days after infection was low (about 10 ID_{50}/ml), virus was not detected in secretions in recovered animals, and therefore mechanical transmission by arthropods or contact transmission would be unlikely from these animals. There is no evidence of recurrence of clinical disease in recovered animals, or of any variation in the level of viraemia although this latter possibility has not been excluded. There is anecdotal evidence that the occurrence of Jembrana disease in Sumatra and Java [8] was associated with the illegal movement of cattle from Bali [unpublished observations], and persistently viraemic recovered cattle were possibly the source of the infection in these areas.

The role played by other animal species in the transmission and perpetuation of JDV is not understood but it is possible they could be involved. It is known that *Bos taurus*, *Bos indicus* and crossbred Bali (*Bos javanicus* × *Bos indicus*) cattle can be infected with JDV and remain viraemic for 3–6 months [3]. Viraemia has also been detected in the Indonesian buffalo (*Bubalus bubalis*) for at least 9 months after infection [6]. Whether JDV could persist in populations of any of these animals in the absence of *Bos javanicus* cattle is unknown.

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