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Viral haemorrhagic disease of rabbits and human health

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SUMMARY

Viral haemorrhagic disease of rabbits (VHD), a potential biological control for wild rabbits in Australia and New Zealand, escaped from quarantined field trials on Wardang Island and spread to the mainland of Australia in October 1995. This study looked for any evidence of infection or illness in people occupationally exposed to the virus. Two hundred and sixty-nine people were interviewed and 259 blood samples were collected. Exposures to VHD-infected rabbits ranged from nil to very high. No VHD antibodies were detected in any of the 259 sera when tested by VHD competitive enzyme immunoassay, which had been validated with 1013 VHDV-specific antibody negative sera. A questionnaire designed to elicit symptoms of disease in a range of organ systems found no significant differences between illness in those exposed and those not exposed to VHD, nor could an association be found between exposure and subsequent episodes of illness. The findings are consistent with the view that exposure to VHD is not associated with infection or disease in humans.

INTRODUCTION

The European rabbit (*Oryctolagus cuniculus*) was introduced into Australia in the 1850s. Since then, it has populated much of Australia and is now regarded as a major agricultural and environmental pest [1]. In 1989, Australia and New Zealand began investigations into the use of rabbit calicivirus (also known as viral haemorrhagic disease virus), the cause of viral haemorrhagic disease of rabbits (VHD), as a possible biological control agent for wild rabbits. VHD was first recognized in China in 1984 following the introduction of rabbit stock from Germany [2]. It spread rapidly in China, covering 50000 square kilometres in less than 9 months and killing 470000 rabbits in the first 6 months [3, 4]. VHD subsequently spread into Asia and Europe and to some American and African countries, killing millions of rabbits [5]. VHD has now been reported in over 40 countries.

Viral haemorrhagic disease virus (VHDV) was imported into the microbiologically secure Australian Animal Health Laboratory (AAHL) in 1991, where testing commenced into its efficacy for killing wild rabbits and for its species specificity. AAHL found no evidence that VHDV could infect any species other than the European rabbit, confirming work in other countries [6–9]. Following these promising laboratory studies, field trials commenced on Wardang Island, South Australia (SA) in March 1995. In September 1995, VHDV escaped from quarantine. Despite the implementation of contingency plans to eradicate the

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disease, the virus continued to spread rapidly across South Australia and was reported interstate by December 1995. Because of the large areas involved and the anticipated costs, plans to eradicate the disease were abandoned.

Despite the escape, assessment of the deliberate release of VHD continued under the Commonwealth *Biological Control Act 1984*, a process that requires public consultation. One of the main concerns raised was the perceived potential for VHDV to infect species other than rabbits, including humans. While there was no evidence in the scientific literature of animals other than the European rabbit being infected, there were also no reports of scientific studies into the human health effects of the virus. Consequently, a Human Health Study Group was formed to plan and manage a study to determine if there were any links between exposure to VHD and serological and clinical evidence of infection with the virus in humans.

METHODS

The study was undertaken in July 1996 and involved serological testing and a questionnaire survey of people with occupational exposure to VHDV. Advice from overseas groups and laboratories working with the virus was also sought.

Study subjects

Participants were mostly government employees working in animal health laboratories, wildlife management or agriculture. Most exposures to VHD occurred in field and laboratory staff involved in the unsuccessful eradication campaign in South Australia during October–November 1996, and those in contact with wild rabbits in areas where VHD was active. More recent exposures occurred in field staff located in the south east of South Australia and in Victoria where the disease was first reported in March 1996.

Government agencies assisted by providing lists of staff. People were approached by telephone and asked to participate in a study to investigate potential links between exposure to wild animals and health. Participation was voluntary. Refusals occurred on the grounds of disliking blood sampling, travel time, distance, or lack of interest. Trained nurses gave the participants a written explanation of the study, an information sheet and obtained a signed consent form before taking blood and completing a questionnaire. In order to increase participation, nurses went to over 30 places throughout the 2 states and sampled participants in their workplace or in various laboratories.

The study was approved by the Ethics Committee of the Royal Adelaide Hospital of South Australia.

Serology

Approximately 20 ml of whole blood was collected from each participant. In South Australia, sera were separated at local laboratories and transported directly to AAHL for serological testing. Victorian specimens were sent to Victorian Infectious Diseases Reference Laboratory (VIDRL) for serum separation and subsequent referral to AAHL.

A competitive enzyme immunoassay (EIA) for VHDV-specific antibody, the most sensitive and specific test for VHDV antibody in rabbit sera [10], was chosen as the screening test for the study. This EIA uses purified whole VHDV particles as antigen, and uses rabbit antiserum, hyperimmune to VHDV, and normal rabbit serum as positive and negative controls respectively. The assay was performed as described [10]. Results were expressed as percentage inhibition values determined by the formula: % inhibition $= 100 \times [1\text{-}(\text{OD test serum}/\text{OD negative control})]$. All sera were tested in duplicate and the mean of the two values was recorded. Sera with a percent inhibition of greater than 50% were considered to be positive, between 30% and 50% was considered to be equivocal, and less than 30% was considered to be negative to VHDV antibodies. Testing was performed blind by laboratory staff.

Under the testing protocol (Fig. 1) any reactors in the competitive EIA were to be tested in a second indirect EIA [10] to clarify their serostatus.

Prior to their use in the study, the specificity of both EIAs was evaluated using a panel of 1000 metropolitan blood and tissue donor sera predating the presence of VHDV in Australia. An additional 13 human sera containing specific antibody to the human viruses most closely related to VHDV, the Norwalk-like group of viruses and hepatitis E virus, were also tested to exclude cross reactivity attributable to these antibodies. These sera were obtained from the VIDRL reference serum collection where they had been maintained at -20 °C. The competitive EIA was evaluated using all 1013 samples of this panel of sera. A more limited evaluation of the indirect EIA was performed using 200 of these sera. Assay sensitivity

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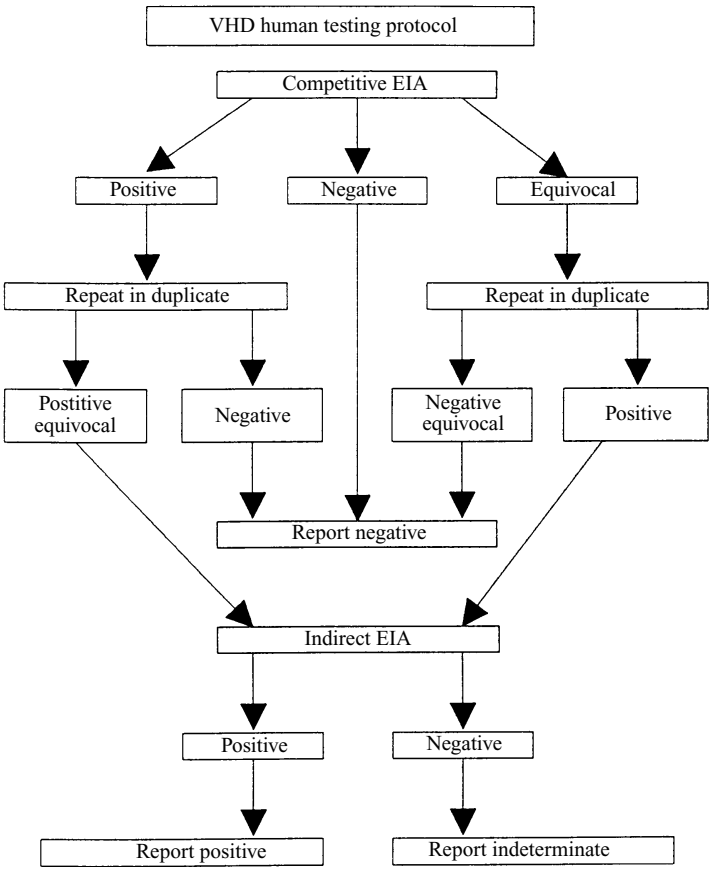

Fig. 1. VHD human testing protocol.

for human serology could not be assessed, since no humans were known to have been infected with VHDV.

International survey

As part of this study, a range of international laboratories and groups working with VHDV were contacted. Information was sought about the consequences of parenteral or mucous-membrane exposure to VHDV-infected material, as well as any evidence of human infections.

The questionnaire

A questionnaire was used to collect information on exposures to rabbits and health status, including illnesses, over the period July 1995 to July 1996, as well as alcohol intake, age, sex, occupation and level of education. In order to reduce possible recall bias (and to avoid unnecessary concern in those exposed to VHDV), potential links between VHD and human illness were not mentioned, questions on health were asked before questions relating to exposure, and participants were first asked about exposure to a variety of Australian wild animals before being asked more specific questions about exposure to rabbits.

The questionnaire sought symptoms associated with hepatic, gastrointestinal and coagulation disorders in view of the natural history of VHD in rabbits [8, 11] and the symptoms in humans of the related Norwalk-like and hepatitis E viruses. Because of the unknown nature of any infection in humans, the range of symptoms under investigation was extended by testing for possible viral effects on other body systems. A set of 26 symptoms was used, grouped into six illness categories (Table 4). For each bout of illness in the study period, the symptoms, the onset date and period of illness was determined as accurately as possible, often with the help of diaries.

Symptoms and illnesses associated with non-infectious causes, such as surgery, diabetes or nausea due to pregnancy and previously-occurring chronic or previously-investigated problems such as long standing dermatitis, asthma or symptoms associated with a previously diagnosed Ross River virus infection, were excluded. Because alcohol consumption is associated with hepatitis, alcohol consumption was also

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Table 1. *Types of contact with rabbits and number of exposures to VHDV*

Type of contact with rabbits	Number of reports of contacts with rabbits		Exposure to VHDV	Number of people with this as the highest level of exposure
	VHD present	VHD not present		
No contact with rabbits	—	—	Nil	118
Ripped warrens, poisoned or fumigated rabbits; handled, cut open or skinned rabbits with a plastic barrier e.g. gloves	91	250	Low	43
Handled, cut open or skinned rabbits with bare hands	115	231	High	108
	206	481		269

measured as a potential confounder of any relationship between exposure to VHDV and hepatitis.

Level of exposure to VHDV

For each person, exposure to VHDV was determined by measuring as accurately as possible the time, location, type of exposure to rabbits and the number of rabbits contacted for each encounter during the period July 1995 to July 1996. A series of detailed maps, documenting the presence of VHD at various times, were prepared from laboratory-confirmed reports, held by the South Australian Animal and Plant Control Commission (J. Kovaliski, personal communication 1996). On the basis of these maps, participants who reported that they had handled rabbits in areas where VHD was known to have been present at the time, were considered to have been exposed.

The level of exposure (Table 1) was defined in terms of contact with rabbit body fluids, since VHDV appears to be transmitted from one rabbit to another by contact with excretions or body-fluids [12]. Thus, exposure was classified as high for subjects who reported skin contact with the body fluids of an infected rabbit, and low for those who reported contact with VHDV-infected rabbits, but did not have contact with their body fluids. Shooting rabbits without picking-up the remains was not considered to be an exposure. Rabbits, when eaten, were all well-cooked, so eating rabbits was also not considered to be an exposure.

Data were analysed using direct analysis and the statistical packages EpiInfo version 6.04 [13] and SAS version 6.03 (SAS Institute Inc. Cary, NC, USA).

RESULTS

Questionnaires were completed by 269 people, 168 people from South Australia and 101 from Victoria. Blood could not be sampled from 10 of these, resulting in 160 and 99 blood samples from each State respectively.

Serology

There were two responses in the equivocal region (percent inhibition 30–50%), but no positive reactivity (> 50%), in the competitive EIA on testing the evaluation panel of 1000 negative sera and the 13 sera containing antibodies to Norwalk-like and hepatitis E viruses (Fig. 2). No reactivity was observed on testing 200 of these 1000 negative sera in the indirect EIA (not shown). Both assays were accordingly used to test the 259 study sera without modification of the assay protocols used for rabbit sera.

None of the 259 test sera were positive, or gave reactivity in the equivocal region in the competitive EIA (Fig. 3). Moreover, the distribution of competitive inhibition values for these sera was comparable to the 1013 presumed VHDV-negative sera. A significant difference in percentage inhibition values was noted between South Australian and Victorian sera (Table 2, Fig. 3), possibly reflecting variations in processing between the states, prior to testing. There was no relationship between exposure status and percentage inhibition values for either set of sera (Table 2).

Although the absence of any reactors obviated the need for supplementary testing, all 259 test sera were also tested using the indirect EIA in case factors unique to the competitive EIA format had contributed to false negativity. All samples tested negative. In

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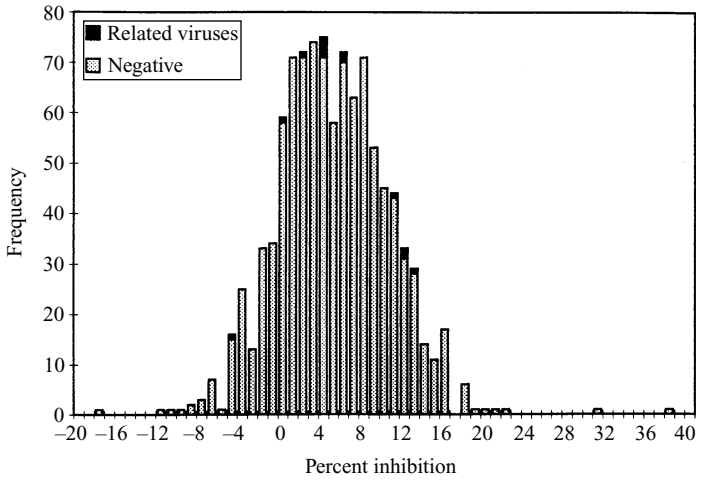

Fig. 2. Frequency distribution of the serological results by competitive EIA for the 1000 negative sera and the 13 sera that were positive for Norwalk-like antibodies and hepatitis E antibodies.

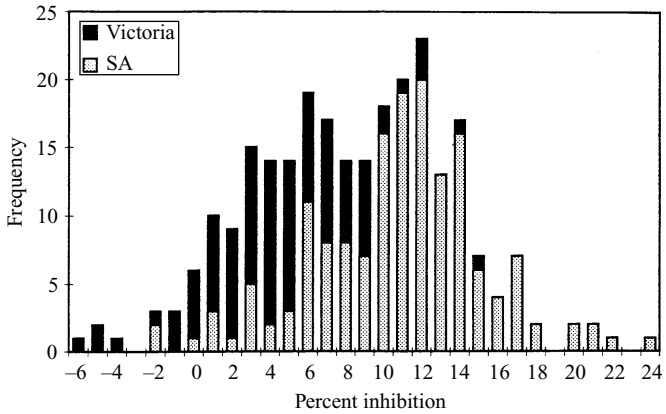

Fig. 3. Frequency distribution of the 269 test sera by competitive EIA.

Table 2. *Serological results*

		South Australia			Victoria			Difference between exposure categories by ANOVA (*P* values)		
	Exposure to VHD	Number	Mean	S.D.	Number	Mean	S.D.	Within SA	Within Victoria	Between states
Competitive EIA	None	67	11·12	4·45	44	3·91	3·93			
Percent inhibition	Low	35	10·60	4·03	5	7·20	4·60			
	High	58	10·53	4·99	50	4·82	3·82			
								0·75	0·10	< 0·001
Indirect EIA	None	67	0·06	0·02	44	0·05	0·02			
Optical density	Low	35	0·07	0·03	5	0·06	0·06			
	High	58	0·07	0·03	50	0·05	0·03			
								0·16	0·53	0·001

addition, optical density values did not relate to exposure status (Table 2). While a scattergram of competitive EIA and indirect EIA results (not shown) showed a linear relationship between the two, the highest values for each test were obtained by different sera.

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Table 3. *Number of episodes of illness for all respondents and date of first exposure to VHD by month*

Number of respondents with first exposure to VHD	Month of exposure	Episodes of illness						
		Any	Diarrhoea	Fever	Rash	Bleeding	Hepatitis	Neurological
38*	Jul 1995	24	2	23	0	0	0	3
3	Aug	23	3	20	1	0	0	3
2	Sep	15	7	14	1	1	0	3
52	Oct	23	10	20	0	0	0	3
9	Nov	19	11	11	1	0	0	4
3	Dec	10	6	7	1	0	0	3
7	Jan 1996	15	6	13	0	1	0	4
8	Feb	14	6	12	0	0	0	3
6	Mar	21	9	20	0	0	0	5
7	Apr	35	17	30	2	0	0	8
5	May	29	11	25	0	1	0	6
10	Jun	64	18	57	7	1	1	15
1	Jul	42	10	40	1	0	1	9
	Unknown	11	6	10	2	1	1	2
151	Total	345	122	302	16	5	3	71

* Including or before July 1995.

International survey

Responses were received from 47 groups in 16 countries. There were no reports of VHDV transmission to humans despite prolonged or repeated exposure to material containing high titres of VHDV. These exposures included a worker with multiple splashes of infected rabbit blood in the mouth and three descriptions of VHDV-contaminated sharps injuries – on multiple occasions in two cases. No subsequent ill effects were reported. Three respondents had undertaken serological testing of small numbers of their laboratory staff, two respondents on more than one occasion. No positive results were recorded.

Questionnaire results

Of the 269 people interviewed, 118 had no exposure, 43 had low exposure and 108 had high exposure to VHDV. The latter group included 19 people with broken skin on their hands who had cut open or skinned infected rabbits. Rangers, farmers, agricultural advisers and veterinary officers had the highest levels of exposure. Although there was no difference in age or educational level between those exposed and unexposed, there was a significant sex difference ($\chi^2_{(1)} = 33$, $P = < 0{\cdot}001$), with more males than females exposed to VHDV. There was a significant difference in alcohol intake between the exposed and unexposed groups ($\chi^2_{(2)} = 8{\cdot}07$, $P = 0{\cdot}02$), but this difference disappeared when the study population was stratified by sex.

Table 3 shows that the distribution of reported episodes of symptoms by month was not uniform, with a peak in the winter months of June and July, 1996. Table 4 also shows the distribution by month of first exposure to VHD in participants. The exposure figure for July 1995 includes exposures before that date, and the large peak in October 1995 corresponds to the exposures during the eradication campaign following the escape of the virus from quarantine. The average period of observation after first exposure was 8·1 months, although disease information was collected from each participant for the full 13 months.

If VHDV causes illness, a higher rate of symptoms and illness in the exposed groups would be expected, regardless of when the participants were exposed. Accordingly, the first analysis compared illness over the entire 13 month study period for the three groups of exposure (nil, low and high). The 26 symptoms and their frequency of occurrence were put into six specific illness groups, and one non-specific group (Table 4). The number of episodes of illness were first compared between the unexposed and combined exposed categories. The number of episodes in the three exposure categories were then compared. There were no significant differences in the individual symptoms between the exposure categories, except for 'presence of dark urine' and 'difficulty speaking and seeing'. All the cases of the first symptom occurred in the

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Table 4. *Reported illnesses and symptoms from July 1995 to July 1996, by exposure category*

Illness	Exposure level to VHD						*P* values	
Respondents reporting illness Episodes reported Symptoms reported				Nil Per 100 respondents	Low	High		
	Nil	Low	High				Nil *vs.* exposed	Nil *vs.* low *vs.* high
Number of respondents	118	43	108	*100·0*	*100·0*	*100·0*		
Diarrhoea/gastro-intestinal illness								
Respondents reporting illness	42	14	38	*35·6*	*32·6*	*35·2*	0·87	0·96
Episodes reported	60	17	45	*50·8*	*39·5*	*41·7*	0·24	0·49
Diarrhoea	45	13	34	*38·1*	*30·2*	*31·5*	0·33	0·62
Vomiting	13	2	17	*11·0*	*4·7*	*15·7*	0·71	0·19
Nausea	31	9	31	*26·3*	*20·9*	*28·7*	0·97	0·70
Stomach cramps	31	10	19	*26·3*	*23·3*	*17·6*	0·22	0·38
Blood in stools	5	1	2	*4·2*	*2·3*	*1·9*	0·29	0·56
Fever/'flu-like symptoms								
Respondents reporting illness	83	27	80	*70·3*	*62·8*	*74·1*	0·96	0·76
Episodes reported	145	45	112	*122·9*	*104·7*	*103·7*	0·15	0·35
Fever	75	30	63	*63·6*	*69·8*	*58·3*	0·84	0·71
Sweats or chills	70	24	62	*59·3*	*55·8*	*57·4*	0·80	0·96
Unusual tiredness or 'off colour'	120	33	91	*101·7*	*76·7*	*84·3*	0·09	0·22
Swollen glands	49	13	31	*41·5*	*30·2*	*28·7*	0·09	0·23
Flu-like illness	119	34	86	*100·8*	*79·1*	*79·6*	0·06	0·18
Sore red or weepy eyes	21	3	16	*17·8*	*7·0*	*14·8*	0·27	0·29
Sore joints or muscles	65	15	47	*55·1*	*34·9*	*43·5*	0·10	0·20
Rashes/skin conditions								
Respondents reporting illness	5	2	6	*4·2*	*4·7*	*5·6*	0·69	0·90
Episodes reported	7	2	6	*5·9*	*4·7*	*5·6*	0·83	0·95
Unusual rashes or blisters on skin	7	2	6	*5·9*	*4·7*	*5·6*	0·83	0·95
Bleeding problems								
Respondents reporting illness	1	0	2	*0·8*	*0·0*	*1·9*	0·71	0·58
Episodes reported	1	0	3	*0·8*	*0·0*	*2·8*	0·45	0·34
Unusual bruising	0	0	0	*0·0*	*0·0*	*0·0*	n/a	n/a
Unusual bleeding	1	0	3	*0·8*	*0·0*	*2·8*	0·45	0·34
Hepatitis symptoms								
Respondents reporting	1	1	1	*0·8*	*2·3*	*0·9*	0·71	0·71
Episodes reported	1	1	1	*0·8*	*2·3*	*0·9*	0·71	0·71
Yellow skin or eyes	1	1	1	*0·8*	*2·3*	*0·9*	0·71	0·71
Neurological symptoms								
Respondents reporting illness	24	9	21	*20·3*	*20·9*	*19·4*	0·93	0·98
Episodes reported	31	15	28	*26·3*	*34·9*	*25·9*	0·73	0·60
Dizziness	28	12	25	*23·7*	*27·9*	*23·1*	0·90	0·86
Blacking out	1	0	1	*0·8*	*0·0*	*0·9*	0·86	0·82
Incoordination, difficulty walking	8	3	6	*6·8*	*7·0*	*5·6*	0·79	0·92
Difficulty speaking or seeing	0	5	2	*0·0*	*11·6*	*1·9*	0·02	0·0002
Fits	0	0	0	*0·0*	*0·0*	*0·0*	n/a	n/a
Weakness or tingling in limbs	4	1	5	*3·4*	*2·3*	*4·6*	0·81	0·78
Non-specific symptoms								
Dark urine	12	0	0	*10·2*	*0·0*	*0·0*	0·00	0·0005
Pale stools	3	0	1	*2·5*	*0·0*	*0·9*	0·21	0·42
Loss of appetite	64	17	42	*54·2*	*39·5*	*38·9*	0·07	0·19
Headache	94	26	74	*79·7*	*60·5*	*68·5*	0·20	0·38
Any illness								
Respondents reporting illness	90	30	82	*76·3*	*69·8*	*75·9*	0·84	0·91
Episodes reported	166	52	127	*140·7*	*120·9*	*117·6*	0·11	0·28

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Table 5. *Expected episodes of illness and exposure to VHD, taking into account different periods of observation in the exposed and unexposed groups*

	Total (number)	Non-exposed (number)	Exposed (number)		*P* value ($\chi^2_{(1)}$)	Non-exposed (standardized)	Exposed (standardized)
			Observed	Expected			
Person months of observation	3497	2129	1368			*100·0*	*100·0*
Number of episodes							
Any	334	204	130	130·66	0·94	*9·58*	*9·50*
Diarrhoea	116	67	49	45·38	0·49	*3·15*	*3·58*
Fever	292	177	115	114·23	0·92	*8·31*	*8·41*
Rash	14	7	7	5·48	0·40	*0·33*	*0·51*
Bleeding	4	1	3	1·56	n/a	*0·05*	*0·22*
Hepatitis	2	0	2	0·78	n/a	*0·00*	*0·15*
Neurological	69	36	33	26·99	0·14	*1·69*	*2·41*

unexposed group. All the cases of the second symptom came from the two exposed categories, but three episodes were from the same person, with the first of these episodes occurring 2 months before that person's first low level exposure to VHDV. After discounting this person, there was no significant difference between the groups.

There were also no significant differences in the numbers of different illnesses reported nor in the numbers of people reporting illnesses, in the three exposure categories. For those respondents reporting episodes of illness, the nil exposure category experienced an average of 1·85 episodes of illness per person, compared to 1·73 episodes per person and 1·54 episodes per person in the low and high exposure categories respectively.

A further analysis was done to see if there were more illnesses immediately following exposure. We would have liked to have considered the number of illnesses in the 30 days following exposure to VHDV, but the dates obtained were not precise enough for such an analysis. Indeed 11 episodes of illness had insufficient date information and had to be excluded. Each bout of illness was allocated to the exposed group if the respondent had been exposed to VHDV in that or a previous month, otherwise it was allocated to the unexposed group. Table 5 shows that the period of observation for exposed and non-exposed respondents was 1368 person months (39·1%) and 2129 person months (60·9%) respectively. An analysis that took these proportions into account found no significant difference in the number of episodes of illness between the exposed and non-exposed groups (Table 5).

Because seasonal variation of disease over the study period (as noted in Table 3) can cause a significant result in such an analysis, episodes of illness were also examined more closely with a series of contingency tables on a month-by-month basis. For each month, respondents were categorized in two ways: whether they were exposed to VHD that month and whether they reported symptoms of illness in the period of that and the following month. This eliminates any seasonal effect, provides a follow-up period of 4–8 weeks after reported exposure and increases the sensitivity of finding an association between exposure and illness. If there was an effect, an increase in the amount of illness over the 2-month period would be expected. However there would also be a correlation between the successive monthly analyses because the corresponding 2-month periods for counting illnesses overlap. No association between exposure to VHDV and illness in humans was found.

DISCUSSION

This study looked for links between occupational exposure to VHDV-infected rabbits and illness in humans. The study had two parts, a serological survey of the study participants to determine if antibodies to VHDV could be detected and an epidemiological investigation into links between exposure to VHD and clinical symptoms of illness.

VHDV-specific antibody was selected as a marker of VHDV infection because it is a persistent marker of past infection in rabbit survivors of VHD. Rabbits maintain detectable levels of antibodies for at least 130 days after infection [10]. Infections with human

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caliciviruses also give rise to long-lasting serological reactions [14].

No VHDV-specific antibodies were detected in any sera using either of the competitive EIA or the indirect EIA. The competitive inhibition values and optical density readings were all well below the respective positive assay cut-offs, and below the assay equivocal zones. These assay thresholds were developed using rabbit sera, and in the absence of sera from VHDV-infected humans, their validity from human serology cannot be proven. There was, however, no relationship observed between VHDV exposure status and serological results, and the competitive inhibition values from the 259 test sera fell into a homogeneous normal distribution, comparable to that of the 1013 control sera used for essay validation. There was, therefore, no evidence of a potential positive population of sera poorly differentiated from negatives by the assays employed.

These results support the findings of the survey of groups working with VHDV in other countries. No respondent reported any case of human infection with VHDV and there were no reports of illness associated with exposure to VHD. Prior to this survey, the only known incident of potential human transmission, was an unpublished report of a low, transient antibody response to VHDV in one person in Mexico with a high level of exposure. The person did not get ill (H. C. Lopez, Director General of Animal Health, Mexico, letter to J. G. Murray 24 January 1996). Given our findings, the antibody response is both unlikely and unexpected and further information on the test used, its sensitivity, specificity and repeatability, would be required before the possibility of a test artefact could be eliminated.

For the epidemiological part of the study, it was difficult to determine how to define exposure to a virus that was not known to transmit to humans and how to measure any resultant clinical illness, when illness in humans had never been described. Exposure categories were assigned on evidence that transmission between rabbits occurs by contact with body fluids [12]. Potential illness was assessed by considering a range of symptoms, including general symptoms associated with viral infections, symptoms observed in rabbits with VHD, and symptoms of humans infected with related viruses. A variety of analyses were done with these data, comparing illness to exposure over the entire study period and on a month-by-month basis. No increase was found in the amount of illness reported in exposed compared to non-exposed people.

A recent review paper on caliciviruses [15], suggests that VHDV may pose a possible threat to humans, citing as evidence data from the initial limited-distribution report of this study [16]. However, the authors' use of the data is misleading. They did not mention the conclusions of the study and chose to classify exposure on the amount of virus in the environment. They noted that the number of cases of illness in the period July–December 1995 was less than in the period February–July 1996 (see Table 3), and argued that during the first period there was a low level of virus, and during the second period, because of the spread of the disease, a high level of virus. They did not consider the actual exposure level of the respondents, and more importantly, that many of the respondents in the earlier period were exposed during the initial attempts to contain the disease (as shown in Table 3). Since the initial report [16], the data have been thoroughly rechecked, the results given here differ slightly, but the conclusion remains the same – there were no significant difference between levels or types of illness in those exposed and those not exposed to VHDV.

In this type of study, there is always the potential that clinical interviews may not detect sub-clinical infections and that the brevity of the interview and its timing, months after some exposures, may diminish the likelihood of identifying minor symptoms. While the absence of detectable antibodies in exposed humans strongly suggests that infection has not taken place, no information is available about whether antibodies may be produced in infected humans anyway, nor the duration of any such response. Without human sera containing VHDV-specific antibody to verify the sensitivity of the assay, it is not possible to definitively exclude the presence of VHDV antibody in sera. In addition, the small sample size in the study make it difficult to exclude the possibility of rare or infrequent infections. These limitations notwithstanding, the study presents a range of data consistent with evidence from other countries, that VHDV is not associated with infection or disease in humans.

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REFERENCES

1. Williams CK, Parer I, Coman BJ, Burley J, Braysher ML. Managing vertebrate pests: Rabbits. Bureau of Resource Sciences and CSIRO Division of Wildlife and Ecology, Australian Government Publishing Service, Canberra, 1995.
2. Liu SJ, Xu HP, Pu PQ, Quian NH. A new viral disease in rabbits. Animal Husbandry Vet Med 1984; **16**: 253–255.
3. Gregg DA, House C, Meyer R, Berninger M. Viral haemorrhagic disease of rabbits in Mexico; epidemiology and viral characterization. Rev Scient Techn Off Intern Epizoot 1991; **10**: 435–51.
4. Xu W. Viral haemorrhagic disease of rabbits in the People's Republic of China: epidemiology and virus characterization. Rev Scient Tech Off Intern Epizoot 1991; **10**: 393–408.
5. Rodak L, Smid B, Valicek L. Application of control measures against viral haemorrhagic disease of rabbits in the Czech and Slovak Federal Republic. Rev Scient Techn Off Intern Epizoot 1991; **10**: 513–24.
6. Fan YY, Yu M, Yang HP, et al. The study of domestic rabbit haemorrhagic virus (in Chinese, translated by D. Sun and L. Chang). Shanghai Dom Anim Hosp Corresp/Commun 1987; **4**: 7–9.
7. Wang Y, Ji C, Zhou Y, Sun H, et al. Study on a new virus infection in rabbits – rabbit pest (in Chinese, English abstract). Scient Agricult Sinica 1988; **21**: 73–9.
8. Xu ZJ, Chen WX. Viral haemorrhagic disease in rabbits: a review. Vet Res Commun 1989; **13**: 205–12.
9. Bureau of Resource Sciences. Rabbit calicivirus disease: a report under the Biological Control Act 1984. Bureau of Resource Sciences, 1996, Canberra.
10. Collins BJ, White JR, Lenghaus C, Boyd V, Westbury H. A competition EIA for the detection of antibodies to rabbit haemorrhagic disease virus. Vet Microb 1995; **43**: 85–96.
11. Marcato PS, Benazzi C, Vecchi E, Galeotti M, Della Salda L, Sarli G, Lucidi P. Clinical and pathological features of viral haemorrhagic disease of rabbits and the European brown hare syndrome. Rev Scient Techn Off Intern Epizoot 1991; **10**: 371–92.
12. Cancellotti FM, Renzi M. Epidemiology and current situation of viral haemorrhagic disease of rabbits and the European brown hare syndrome. Rev Scient Tech Off Intern Epizoot 1991; **10**: 409–22.
13. Dean AG, Dean JA, Burton AH, Dicker RC. Epi Info, Version 6.03: a Word processing, database and statistics program for epidemiology on microcomputers. USD Incorporated, Stone Mountain, Georgia, 1996.
14. Kapikan AZ, Greenberg HB, Cline WL, et al. Prevalence of antibody to the Norwalk agent by a newly developed immune adherence haemaaglutination assay. J Med Virol 1978; **2**: 281–94.
15. Smith AW, Skilling DE, Cherry N, Mead JH, Matson DO. Calicivirus emergence from Ocean reservoirs: zoonotic and interspecies movements. Emerg Infect Dis 1998; **4**: 13–20.
16. Human Health Study Group. Rabbit calicivirus and human health. Report of the Rabbit Calicivirus Human Health Study Group, 1996.