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# PREVALENCE OF MOUSE MAMMARY TUMOR VIRUS (MMTV) IN WILD HOUSE MICE (*MUS MUSCULIS*) IN SOUTHEASTERN AUSTRALIA

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ABSTRACT: We determined the prevalence of mouse mammary tumor virus (MMTV) in introduced, free-roaming, wild house mice (*Mus musculis*) and compared envelope (env) and long terminal repeat (LTR) nucleotide sequences of viruses from wild mice and other sources. Mice were trapped on two occasions, in October (spring) and the following May (autumn) of 2003–2004 in the Mallee region of northwestern Victoria, Australia. Animals were assigned to three cohorts (subadult, young, and old adults) based on their body length. The DNA from salivary glands (62 of 62 mice) and mammary glands (19 of 32 female mice) was screened for the MMTV envelope (env) gene, and the long terminal repeat (LTR) region including the superantigen (SAg) sequence was amplified from a subset. Positive polymerase chain reaction (PCR) results for the MMTV env PCR were detected from salivary gland tissues from 60 of 62 (97%) mice and from mammary gland tissues from 19 of 19 (100%) female mice. All but two mice were positive for MMTV env across both sexes and the three cohorts. Similarity of the SAg carboxy-terminal nucleotide sequence between free-roaming wild house mice varied from 64% to 99%, although most of this variation was due to DNA sequences from two mice (M4 and M5). Phylogenetic analysis of the LTR region did not result in distinct grouping of sequences derived from mice when comparisons were made among sequences from mice in the US, Europe, and Australia, and MMTV-like virus (MMTV-LV) env sequences derived from human hosts. We report a high prevalence of the MMTV env sequence during a sampling period when peak mouse density was low. This indicates that MMTV is an enzootic virus in a population of wild, free-ranging mice in northwestern Victoria, in Australia. Phylogenetic analysis, based upon env and LTR sequence data, indicated minor variation among all isolates. This represents the first report on the prevalence of MMTV in mouse populations in Australia.

*Key words:* Australia, long terminal repeat, LTR, MMTV, mouse mammary tumor virus, *Mus musculis* PCR, wild house mice.

## INTRODUCTION

In Australia and elsewhere, populations of the introduced house mouse (*Mus musculis*) can increase to very high numbers. Widespread population increases (mouse plagues) of house mice occur at irregular intervals in Australia (Singleton et al., 2005). Mice in wild and domestic habitats are infected with a range of viral pathogens, including mouse mammary tumor virus (MMTV), and we postulated that this virus was present in wild house mice in Australia.

Mouse mammary tumor virus is a nontransforming retrovirus that causes breast cancer in mice via insertion of a provirus near a known set of protooncogenes, resulting in upregulation of these genes and tumorigenesis (Cohen, 1979; Nusse and Varmus, 1982; Callahan and Smith, 2000). We and others have also identified MMTV-like envelope (env) sequences in another species (humans) from Australia and the US (Wang et al., 2001; Ford et al., 2003; Etkind et al., 2004; Faedo et al., 2004). Currently, there is no published information on the prevalence of MMTV in free-roaming wild house mice in Australia. Our objective was to investigate the prevalence of MMTV in wild-caught house mice sampled from the same location (northwestern Victoria, Australia) in spring and again the following autumn



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in order to determine prevalence rates. Moreover, we compared the DNA sequences obtained from these free-roaming wild house mice with those from other continents and MMTV-like sequences from humans. This is preliminary to examining the potential for zoonotic transmission of MMTV from mice to humans.

## MATERIALS AND METHODS

Trapping was conducted on two occasions, in October 2003 (spring) and the following May 2004 (autumn), in wheat fields at Walpeup (35°08′S, 142°02′E), northwestern Victoria, Australia. Traps were set in the afternoon and evening and checked soon after sunrise. Each mouse was killed by cervical dislocation, weighed, sexed, and measured for length, and their breeding condition was assessed (females—pregnant, lactating, uterine scars; males—scrotal or abdominal testes). Animals were assigned to three cohorts based on their body length: subadults were <72 mm, young adults were 72 to 77 mm, and old adults were >78 mm (Singleton, 1989). Appropriate organs (mammary glands and salivary glands) were removed from all mice aseptically and immediately placed into uniquely numbered vials that were then stored in liquid nitrogen.

Standard precautions were followed to avoid contamination, including separate rooms for polymerase chain reaction (PCR) preparation, DNA extraction, and DNA amplification. DNA was extracted from tissues using a QIAamp DNA mini kit (cat. no. 51306, Qiagen, Doncaster, Australia), according to the manufacturer's protocol. All samples were screened first for a housekeeping gene (GAPDH), and, if positive, they were then tested for MMTV envelope (env) as described in Ford et al. (2003). In brief, RNA was reverse transcribed with poly (dT) primer, cDNA was subjected to 35 rounds of amplification using primer MMTV1F (sequence CCAGATCGCCTTTAAGAAG, at position 695–714 on MMTV sequence, with Genbank accession number AF43689) with MMTV2R (sequence TACAGGTAGCAGCACTATGG, at position 1269–1289 on MMTV sequence, accession number AF43689). A second set of reactions was performed with primer MMTV3F (sequence TGCGCCTTCCCTGACCAGGG, located at position 762–781 on MMTV sequence, with Genbank accession number AF43689) with MMTV4R (sequence GTAACACAGGCAGATGTAGG, at position 1048–1117 on MMTV sequence, accession number AF43689). The amplified DNA was detected using gel electrophoresis on 1% agarose gels (Ford et al., 2003; Faedo et al., 2004).

The long terminal repeat (LTR) region of MMTV was amplified (Wang et al., 2001), and sequences were compared to published sequences from mice positive for exogenous and endogenous MMTV (Donehower et al., 1983; King et al., 1990; Korman et al., 1992; Pullen et al., 1992; Rudy et al., 1992) and published sequences from another (human) host (Liu et al., 2001; Wang et al., 2001; Ford et al., 2003; Etkind et al., 2004; Faedo et al., 2004). Nucleic acid sequences were aligned using Clustal W, and a tree was constructed from the carboxy-terminal superantigen (SAg) sequences contained within the amplified LTR region using DNApars (ANGIS, 2005; Felsenstein, 1989). This program was used to perform unrooted parsimony analysis, analogous to construction of Wagner trees (Eck and Dayhoff, 1966; Kluge and Farris, 1969).

## RESULTS

Only nine adult female mice were collected early in the breeding season in October 2003. Of these, eight were pregnant and lactating, and one was lactating. No adult males were collected for analysis at this time. In May 2004, 23 females and 30 males were sampled. The May sample was evenly distributed across the three age cohorts of mice for both sexes. Of the 17 adult females, three were pregnant, and eight were lactating. All but two mice were positive for MMTV across both sexes and the three cohorts (Tables 1 and 2). These positives represent endogenous MMTV, exogenous MMTV, or both, since both would contain the env gene amplified by PCR. A segment of the open reading frame coding for the MMTV SAg was amplified from salivary gland tissue of 28 mice (20 males and eight females) positive for MMTV env. The identity of the SAg carboxy-terminal DNA sequence between the wild house mice varied between 64% and 99% (Liu et al., 2001; Ford et al., 2003; Etkind et al., 2004; Faedo et al., 2004). Most of this variation was due to DNA sequences from two mice (M4 and M5). Variation in SAg between

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TABLE 1. Prevalence of the mouse mammary tumor virus (MMTV) in salivary and mammary glands of wild house mice (*Mus musculis*). Mice were sampled in spring and autumn 2003–2004 at Walpeup, northwestern Victoria, Australia.

| | | | GAPDH PCR | | MMTV env PCR | |
|---|---|---|---|---|---|---|
| | No. of mice | Tissue type | No. tested | No. positive (%) | No. tested | No. positive (%) |
| Female | 32 | Salivary | 32 | 32 (100) | 32 | 32 (100) |
| | | Mammary | 19 | 19 (100) | 19 | 19 (100) |
| Male | 30 | Salivary | 30 | 25[a] (83) | 30 | 28[b] (93) |
| Total | 62 | | 62 | 57 | 62 | 60 |

[a] Glyceraldehyde phosphate dehydrogenase negative male mice were MMTV positive as a result of lower limit of detection of MMTV envelope (env) polymerase chain reaction (PCR).

[b] MMTV-negative male mice were GAPDH positive (a housekeeping gene).

mice was 93% to 97% when these two sequences were removed. Four of the 11 published mouse sequences were from strain DBA/2J (an inbred strain with no outlying wildness measurements), and these were 98% to 99% identical in nucleotide sequence in the SAg carboxy-terminal region. When a DNA sequence from DBA/2J was included in the comparison, the five sequences were 64–99% similar. Three DNA sequences were from the strain C3H, and these varied by 71% to 99% from the consensus, but on removing one endogenous isolate, the remaining two exogenous isolates were 99% similar. Overall the 11 published mice DNA sequences varied in SAg nucleotide sequence by 64% to 99%.

The sequences derived from free-roaming wild house mice in Australia did not form a distinct group from those derived from mice in Europe, the US, or those derived from humans. The alignment of published mouse sequences showed that they did not appear to group according to endogenous or exogenous origin, but rather nucleotide sequences obtained from particular strains of mice grouped together. Notably, Mtv-6.1, Mtv-6.2, Mtv-1, and Mtv-13 were all derived from the DBA/2 strain. Likewise, Australian mouse sequences grouped with both endogenous

TABLE 2. Prevalence of mouse mammary tumor virus (MMTV) tabulated for season, sex, tissue, and cohort size in free-roaming wild house mice (*Mus musculis*). Mice were sampled in spring and autumn 2003–2004 at Walpeup, northwestern Victoria, Australia.

| | | | | Prevalence of MMTV across cohorts[a] No. positive (% positive) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Subadult (<72 mm) | | | Young adult (72–77 mm) | | | Old adults (>77 mm) | | |
| Season | Sex | No. positive (%)[a] | Mouse length mean (±SEM) | SG[b] | MG | T | SG | MG | T | SG | MG | T |
| Spring | Female | 9 (100) | 87.7 (1.87) | 0 | 0 | 0 | 0 | 0 | 0 | 9 | 9 | 9 (100) |
| Autumn | Female | 23 (100) | 76.5 (1.27) | 6 | 0 | 6 (100) | 7 | 0 | 7 (100) | 10 | 10 | 10 (100) |
| | Male | 28 (93) | 76.3 (1.40) | 9 | 0 | 9 (100) | 8 | 0 | 8 (100) | 11 | 0 | 11 (85) |
| | Total | 60 (97) | | | | 15 (100) | | | 15 (100) | | | 30 (94) |

[a] MMTV envelope (env) polymerase chain reaction (PCR).

[b] SG = salivary gland tissues, MG = mammary gland tissue, T = total.

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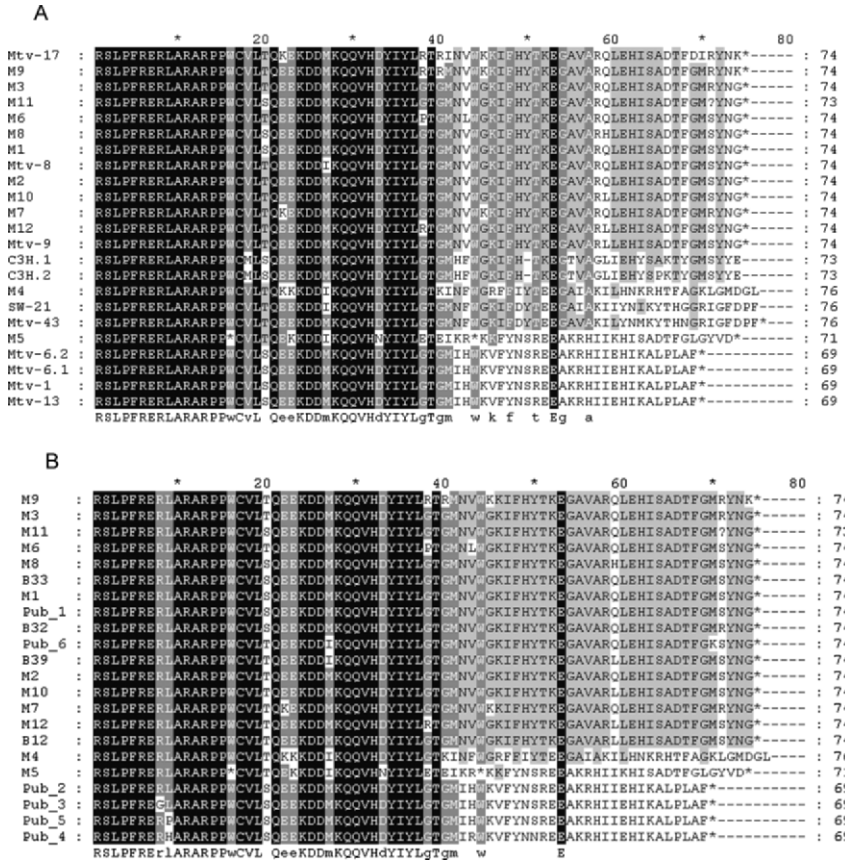



FIGURE 1. Derived protein sequence of the carboxy-terminal end of the mouse mammary tumor virus (MMTV) superantigen from Australian wild house mice aligned with: (A) derived protein sequences from published mice sequences and (B) derived protein sequences from human MMTV-like virus (MMTV-LV) sequences in human breast cancers. Australian wild house mouse sequences: M1—0504002, 0504008, 0504029, 0504034, 0504035, 0504042, 0504044, and 310003; M2—0504011, 0504012, 0504037, 0504049, 0504051, 310002, 310010, and 310014; M3—0504001, 0504006, and 0504045; M4—0504004; M5—0504007; M6—0504010; M7—0504023; M8—0504025; M9—0504026; M10—310005; M11—310008; M12—310009. Published mice sequences: Mtv-1, (DBA/2), X63024 (Pullen et al., 1992) and X64553 (Korman et al., 1992); Mtv-6.1, DBA/2, X63026 (Pullen et al., 1992); Mtv-6.2, DBA/2, X64554 (Korman et al., 1992); Mtv-8, C3H, J02273 (Donehower et al., 1983); Mtv-9, B10.A, M29600 (King et al., 1990); Mtv-13, DBA/2, X63027 (Pullen et al., 1992) and X64555 (Korman et al., 1992); Mtv-17, DBA/2J, X64556 (Korman et al., 1992); Mtv-43, MA/MyJ, X64541 (Rudy et al., 1992); C3H.1, J02274, (Donehower et al., 1983); C3H.2, K00556 (Majors and Varmus, 1983) and SW-21, X65340 (Held et al., 1992). Australian human breast cancers: B12—infiltrating ductal carcinoma (IDC) grade I; B32—IDC grade II; B33—IDC grade II and B39, IDC grade I. Published human breast cancers: Pub1—AY652968 (Etkind et al., 2004) and AF243039 (Liu et al., 2001); Pub2—AY652967 and AY652969 (Etkind et al., 2004); Pub3—AY652964 (Etkind et al., 2004); Pub4—AY652973 (Etkind et al., 2004); Pub5—AY652974 (Etkind et al., 2004); Pub6—AY652977 (Etkind et al., 2004). Note, M11, position 71, had a double peak in the DNA sequence, which results in either arginine or serine, indicated in the alignment by ?. A stop codon is indicated by *.

and exogenous sequences. There was no clustering of Australian isolates, and both Australian wild house mouse (Figs. 1, 2) and human sequences were distributed throughout groups (Figs. 1, 2).

## DISCUSSION

Evidence of MMTV was found in nearly all male and all female mice sampled. Comparison of different MMTV genomes

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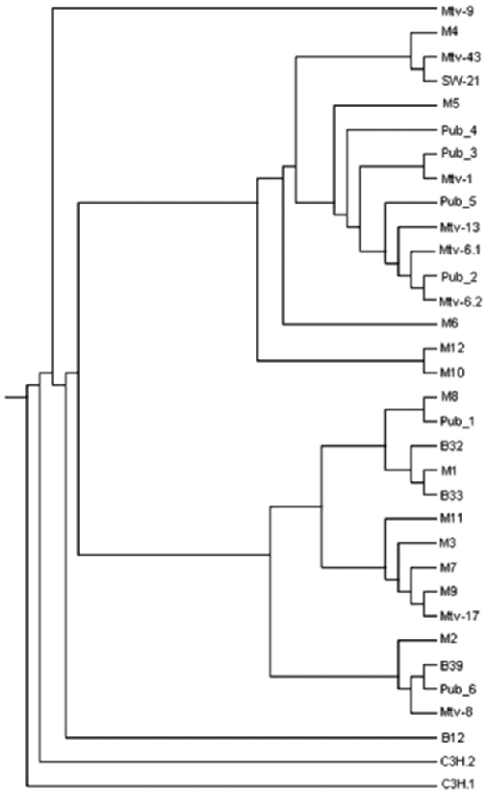



FIGURE 2. Cluster analysis of the derived protein sequence of the carboxy-terminal end of the mouse mammary tumor virus (MMTV) superantigen from Australian wild house mice, including a subset of derived protein sequences from published mouse and human MMTV-like virus (MMTV-LV) sequences in human breast cancers from data in Figure 1.

was based upon sequencing the envelope gene (env) and the long terminal repeat (LTR) regions. The env gene encodes a surface protein subject to host immune selective pressure, and the LTR encodes a superantigen (SAg) under different selective pressure. Despite the difference in the proteins encoded by these two genes, with the exception of two mice, MMTV was fairly homogeneous in env and LTR nucleotide, and derived amino acid sequences.

There is little published information on the prevalence of MMTV in wild house mice, and the majority of investigations have focussed on MMTV in laboratory strains (Callahan et al., 1982, 1986). In this study, we report a high prevalence of MMTV based on detection of the env and LTR sequences in subadult and adult male and female house mice. House mice in wheat fields in this region can have a breeding season as short as 4.5 mo or as long as 10 mo, and peak annual population densities vary from less than one mouse per hectare to more than 1,000 mice per hectare (Singleton et al., 2005). In 2003, peak mouse density was low (<30 mice per hectare; P. Brown and A. Arthur, unpubl. data), and the breeding season lasted 7.5 mo. Under these population conditions, the prevalence of the virus in all samples of mammary and salivary glands of females and in a majority of subadult, young adult, and old adult animals of both sexes indicates that MMTV is an enzootic virus in this population of free-roaming, wild house mice in Victoria, Australia.

Two studies performed in California (USA), used electron microscopy (EM) to detect type B particles in wild caught house mice (Rongey et al., 1973, 1975). The first found that 60% of female mice (25 of 43) were positive for type B particles (thought to represent MMTV) in breast tissue (Rongey et al., 1973). Similar results were noted in 58% (seven of 12) of milk samples, although no type B particles were detected in spleen samples (0 of 35) (Rongey et al., 1973). The second study collected the submaxillary gland of males and females, and it detected type B particles in only 22% (six of 27) of pregnant females (Rongey et al., 1975), and type B particles were not seen in nonpregnant females (zero of seven) or normal males (zero of 14; Rongey et al., 1975). The proportion of positive mice found in both studies is considerably lower than that found in this study. This could be expected since the previous analyses depended on the virus being expressed in the tissues at the time of sampling, and they utilized EM, where we tested using PCR in two tissues. Some of the mice previously studied may have been positive

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for virus DNA but not producing viral particles (Rongey et al., 1973, 1975).

Screening by PCR will detect provirus or free whole virus particles, and hence both endogenous and exogenous MMTV will be found using the methods described here. The carboxy-terminal region of the SAg of MMTV is polymorphic, and some authors have suggested that it can be used to distinguish isolates of endogenous and exogenous origin (Brandt-Carlson et al., 1993). It is not possible to determine if the positives detected in our studies represent active viral infection or endogenous MMTV. It is not clear from published studies if endogenous MMTV is capable of producing retroviral-type particles. Studies from groups in the US and Australia indicate that MMTV SAg and env-like gene products are present in humans (Wang et al., 2001; Ford et al., 2003; Etkind et al., 2004; Faedo et al., 2004). The alignments of env and LTR sequences performed here indicate little variation between nucleotide and derived amino acid sequences from MMTV of mice and MMTV-LV of humans (Figs. 1, 2). Another area of investigation involves determining if wild animal contacts of mice also have MMTV sequences, particularly given that MMTV causes tumors and death of mice (Stewart et al., 2000).

This study indicates for the first time, that MMTV is prevalent in wild-caught house mice in an agricultural area in southern Australia. It is unknown if a similar prevalence occurs in metropolitan regions, and further research to address prevalence of MMTV in urban house mice, and other species contacting (or feeding upon) mice, is required. Epidemiological surveys of the animal host are important because they define the virology of the wild population. We have shown similarities between derived amino acid sequences of MMTV env derived from MMTV populations around the world and MMTV-LV of humans. Although there are currently no data on MMTV-LV in humans as a zoonotic infection, this genetic similarity between MMTV and human MMTV-LV should stimulate further study of this area.

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