**ORIGINAL ARTICLES**

**Gender-disordered children: does inpatient treatment help?**

*(for editorial comment, see page 561)*

**ABSTRACT** Treatment guide-lines for gender-disturbed children currently are unclear. This clinical report describes eight children with cross-gender behaviour who were treated in an inpatient unit for children. The short-term outcome and long-term clinical observations are provided, which indicate a generally good outcome. The findings may have both practical and theoretical significance because they suggest that some gender disorders may be determined by intrafamilial interactions which are correctable.

Robert J. Kosky

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**Princess Margaret Hospital for Children, Thomas Street, Subiaco, WA 6008.**

**Robert J. Kosky, FRANZCP, Director, Psychiatric Services; and State Director, Child and Adolescent Psychiatry Services, Health Department of Western Australia.**

Reprints: Dr R.J. Kosky.

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**Clinical records**

Between 1975 and 1980, eight primary school-aged children with cross-gender behaviours were referred to the Child and Adolescent Psychiatry Services, Perth, Western Australia. Each child was examined by an experienced child psychiatrist; some were examined by me personally. The clinical features that are reported here were taken from psychiatric interviews and the clinical and nursing notes. The salient points are summarized in the Table.

By the term cross-gender behaviour, I refer to the attitudes, affectations, mannerisms and interests that were exhibited by the children as manifestations of the opposite sex. These behaviours included persistently dressing in clothes that are identified as those of the opposite sex and walking, talking and playing in ways that are commonly associated with the opposite sex. The cross-gender behaviours did not usually correspond to the child's age but involved behaviours that are generally associated with adults of the opposite sex; for example, a nine-year-old boy wearing high-heeled shoes, stockings, jewellery and make-up. The cross-gender behaviours sometimes centered on a few activities, such as sitting in front of a mirror and applying make-up for hours on end. Such extended occupations precluded the wide-ranging activities and interests that are characteristic of other children. The children denied their biological sex to a greater or lesser degree. The one female patient vehemently denied her sex and expressed disgust at the genital evidence of it. Others were less marked, but all said they wanted to be of the opposite sex and did not like their genitals. One boy sat to micturate. Other authors have provided similar descriptions of cross-gender behaviour, and the criteria for the classification of gender identity disorder of childhood in the *Diagnostic and statistical manual of mental disorders* (3rd edition) encompass these features.

The unhappiness of these children was noted in the initial psychiatric interviews. Some were noted to have suicidal ideas. Each expressed sadness and loneliness. All were of average or above average intelligence and had no physical abnormalities. There was no evidence of psychosis. Their parents were unhappy, especially the parent of the opposite sex who seemed tied to the home, lonely, and with few social outlets. This parent usually described a close emotional bond between themselves and the child. The parent of the same sex was either absent from the home, away for long periods, or worked excessively long hours. Frequently, a grandparent appeared to be the dominating influence in the household.

In general, cross-dressing began when the child was very young, usually around two years of age. The parent of the opposite sex may have initiated this as a "joke" ("Doesn't he [or she] look lovely?") and, with delight, found that, when the child was dressed in clothes of the opposite sex, play together was fun. The child later cross-dressed on his or her own. This parent and the child undertook few activities outside the home. Most of the children had not attended kindergarten and school refusal had occurred in most cases. When at school, the child's cross-gender behaviour became very noticeable and was remarked upon negatively by teachers and other children. The parents became aware that their child had failed to develop age-appropriate peer relations or social skills and was friendless and unhappy.

Psychological assessments, including the "Draw a Person" Test and the Irt-Scale for Children**, confirmed the clinical findings that these children denied or rejected their biological sex and identified with the opposite sex.

**Case 1**

David (a pseudonym), who was aged six years (IQ = 122), refused to go to school unless he were dressed in female clothes. When he arrived at school, after his mother had agreed to his demand, he was scorned by the other children. He was "friendless, lonely and unhappy, except when playing "dress-ups" at home with his mother. His father was a truck driver and absent from home for long periods; his mother was a shy, introverted woman who had few acquaintances.

David's brother and sister were much older. David's mother had wanted another daughter. After David's birth she was depressed for nearly a year and felt that he was a "stranger". When he was about 18 months old she had dressed him in her daughter's old baby clothes, which she had kept. She had enjoyed this, "it was a joke, really", so she continued to indulge the whim and found that she and David played easily together during "dress-ups". Soon, David dressed himself in his sister's clothes and used his mother's make-up. He attended kindergarten sporadically. When he did, he headed straight for the "dress-up" box and spent his time there in solitary play. Such behaviour was not possible at school, and David's mother quickly became aware that he was isolated socially and incapable of mixing with other children.

**Case 2**

Reginald (a pseudonym) was seven years old (IQ = 105). He was an extremely unhappy child and had thought a lot about suicide. He had preferred girls' clothes since he was two years of age, when he became "attracted" to his older sister's underwear. He preferred to play with girls, but mostly liked to be at home with his mother. He expressed a strong desire to be a girl and preferred to sit to micturate. Normally he was uneasy and withdrawn in any social situation, but when dressed as a girl he showed off, affecting "girlish" mannerisms. The children at school ignored him and the teachers reported that he could cope only in a one-to-one situation.

Reginald was unplanned and born when his parents' marriage was in a bad state. His mother told us that from the time Reginald was born he was "bullied" and "dominated" by his older sister who would often say "if only he was a girl". However, after clinical observations of the family...
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TABLE: Clinical features and progress

<table>
<thead>
<tr>
<th>Case no.</th>
<th>Age (years) and sex</th>
<th>Cross-gender behaviours</th>
<th>Other problems</th>
<th>Treatment length (weeks)</th>
<th>Follow-up at one year</th>
<th>Age last seen (years)</th>
<th>Postpubertal status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6M</td>
<td>Dresses in mother's and sister's clothes</td>
<td>School refusal. Enuresis</td>
<td>13</td>
<td>No cross-gender behaviour</td>
<td>16</td>
<td>No cross-gender behaviour. Male identified. Heterosexual erotic attraction &quot;Quite well&quot;, has &quot;ups and downs&quot; in mood. Relates to both sexes. Erotic desires unknown. Parents' marriage &quot;great&quot;</td>
</tr>
<tr>
<td>5</td>
<td>10M</td>
<td>Wears female under-clothes. Wants to be a girl</td>
<td>Unhappy. Friendless. Lacks initiative</td>
<td>17</td>
<td>Happy, making friends. Involved in sport. No cross-gender behaviour. Some antisocial traits</td>
<td>18</td>
<td>No further contact</td>
</tr>
<tr>
<td>6</td>
<td>10M</td>
<td>Cross-dresses. Effeminate. Objects to being male</td>
<td>Unhappy. Friendless</td>
<td>6</td>
<td>Discharged against medical advice</td>
<td>19</td>
<td>Homosexual activity between ages of 14 and 17 years. Unhappy about this. Sought therapy. &quot;Feels heterosexual&quot; but no heterosexual activity yet. Very successful career</td>
</tr>
</tbody>
</table>

It was considered that the mother was projecting her own feelings into her description of her daughter's behaviour.

Reginald's mother was a lonely woman. Her husband spent much time at work or "out with the boys", and although he was superficially charming, he seemed an insecure man much dominated by his own mother. Both he and his wife loved one another, but both were immature and frightened of the opposite sex (later they benefited from sex therapy).

**Case 3**

Kevin (a pseudonym) was an eight-year-old boy (IQ = 120) who had insisted on wearing women's clothes since he was about two years old. There were grave problems after his birth because his mother developed a postpartum psychosis. Kevin suffered some bizarre and hurtful punishments at her hands during this period. On one occasion she bit through the flesh around his thumb. After 18 months, when she recovered, his mother was concerned about Kevin and felt guilty. During this time, when he dressed as a girl, she could play happily with him. His father was never involved, left the family when Kevin was aged four years, and had rarely liked to see his son. After a long financial battle, Kevin's mother had finally achieved some stability. However, Kevin's behaviour had become a social embarrassment to her at school and at home. She said "every time there's a man around, he [Kevin] wiggles his bum!"

Kevin was a withdrawn, unhappy child, aggressive to his sister, friendless, who performed poorly at school, and cross-dressed whenever he could.

**Case 4**

Dorothy (a pseudonym) was 10 years old (IQ, superior range) when she was referred because of crying, unhappiness, complaints of aches and pains with no organic cause, and school refusal. She refused to wear her girls' school uniform and dressed only as a boy. At school she ignored girls and would, unsuccessfully, attempt to mix with boys. Although very bright, she avoided female teachers and refused to learn from them, thereby creating educational difficulties. She had a strong objection to being female and an overt desire to be a boy.

Her parents had separated two years earlier. Her older brother and sister lived with her father, while Dorothy lived with her mother. Her mother had objected to Dorothy going to live with her father because of his "influence". He had always wanted Dorothy to be a boy, had treated her as one and called her "Rocky". Dorothy's father made few efforts for his family, except for Dorothy. Her father had first bought male clothes for Dorothy when she was very young and usually gave her male attire or masculine games for birthday presents.

Dorothy's father accompanied her to the hospital. Dorothy was dressed in football clothes, including spiked football boots, and was carrying a football that her father had given her recently.

**Cases 5-8**

Patients 5 (IQ = 107), 6 (IQ = 106), 7 (IQ = 120) and 8 (IQ = 112) were four boys, aged 10 to 12 years. Their clinical features are summarized in the Table. Each cross-dressed and each was unhappy, the eldest child being quite suicidal at the time of his admission to hospital. Each was dominated by their mother and received little or no support from their father. In Cases 6, 7 and 8, the mothers had actively encouraged the children in interests in female clothes, jewellery and fashions. None of these children had friends, and all were failing to progress academically at school.

From these histories it becomes apparent that the cross-gender behaviour was not the only, or indeed the central, problem for most of the children. Unhappiness, anxiety, suicidal thoughts, aggressiveness and failure to learn adequately at school were features that were present in most. As were the cross-gender behaviours, these features appeared to be secondary to the pathological parent-child relationships.
Formulation and therapeutic goals

The essential disturbance in these cases was the inability of the parent of the opposite sex to accept the child, except on the conditional basis that the child would meet certain of their needs. The parents sought companionship from the child, free of the anxiety that was created in them by gender differences. The parent, to overcome his or her anxiety, developed a fantasy about the child. He or she denied the child's biological sex, and encouraged the child to adopt cross-gender behaviour in their child. When the child adopted these behaviours, the parent changed from a cold, mechanical interaction with the child to warmth and affection. In a few cases, overt rejection of the child had occurred in the early years, only to be compensated for when the child was "recreated" in the clothes of the opposite sex. Naturally, the child sought to maintain and extend the new relationship, so, spontaneously, adopted the behaviours that were desired of him or her.

Such a symbiotic relationship between parent and child, while possibly fulfilling the parent's emotional needs, avoided the anxiety of the child to accommodate the parent's needs. The parent needed to increase social competence, to improve their self-confidence by ordinary social skills, reinforcing the dyadic relationship, and to develop interests and activities such as tennis, swings or jumps, and by developing friendships with other children.

The parent of the same sex was also socially impoverished, not one who could facilitate the child's developmental maturation, especially since the opportunity for "mucking around" with other children was denied and the behaviours cut the child off from his or her peers. In both parent and child, the mutually sustaining relationship precluded the development of ordinary social skills, reinforcing the dyadic dependence.

The parent had focused attention on the child because their own life was so unfulfilling, and the repertoire of their social skills relatively barren. The parent of the same sex was also socially impoverished, and avoided the anxiety of the child to accommodate the parent's needs. The parent needed to increase social competence, to improve their self-confidence by mastering age-appropriate social relationships and activities, and to develop interests and activities to allow their own unique personalities to mature. To meet these needs, the warm encouragement and affectionate support, and their parents were not one who could facilitate the child's developmental maturation, especially since the opportunity for "mucking around" with other children was denied and the behaviours cut the child off from their own unique qualities, and to demonstrate this in mutually enjoyable, developmentally appropriate play with the child.

The choice of inpatient treatment was determined by the location of the primary problem in the family interactions and the unwitting, but pathological, influences of the parents. In addition, the need to develop the child's social skills so that he or she could make friends and cultivate resources outside the home seemed imperative.

The child's needs, it was felt, could best be met through day-to-day involvement in a therapeutic environment that was provided by the inpatient unit,19 while those of the parents could be tackled during the times of their visits and by special outpatient appointments with psychiatrists and social workers. The treatment programme

With their parents' permission, the children were admitted to Stuyvesant Terrace Hospital, a free-standing psychiatric unit for children, at different times between 1975 and 1980. Only one gender-disordered child was in the unit at any one time. The other children in the unit had a range of disorders including neurotic illnesses (depression, anxiety, conversion reactions, compulsions, and so on), psychoses, problems (anorexia nervosa, eczema, asthma), adjustment reactions or reactions to chronic illnesses. None was delinquent or intellectually handicapped. Most were well enough to attend the local primary school each day and they joined in many play activities together.

At school and in the hospital, all children were encouraged to join in games with other children. A wide range of activities was provided at the hospital and the child chose what interested him or her. No conscious attempt was made by the staff to encourage masculine or feminine role behaviours. The only prohibition that was placed on boys who cross-dressed was that they must respect the privacy of others and, therefore, not steal girls' underwear.

Age-appropriate behaviours were encouraged by the nursing staff members to replace the stereotyped inappropriate and isolating cross-gender role behaviours. The child was encouraged to join in activities and play with the children. The children went home for some of the weekends, sleeping at home for one night. Parents saw either a psychiatrist or a social worker once a week, as well as the nursing staff members who were assigned to them for their visits, in order to discuss their child's progress and their marital relationships. They were given counselling about how to respond to their child and how to improve their own social life.

Results

Short-term outcome

Cross-dressing ceased very quickly after admission to hospital. Many of the other cross-gender behaviours, which had been present for years, vanished after several weeks. Such dramatic changes in the children's behaviour produced anxiety for all the parents. The mother of Patient 6 had panic attacks. As a result of this, her son then reverted to cross-dressing, wearing jewellery, perfumes, and so on, and she settled down. When he again ceased cross-dressing, she began sabotaging the treatment by bringing in female clothes for him and isolating herself with him in his room. In spite of our efforts she removed her son from the hospital. We have had no further contact with this child. The other seven children remained as patients for between 10 and 23 weeks (average, 18 weeks).

In general, nursing staff members and clinicians reported improvements in the general mood of the child after admission to hospital, although episodes of misbehaviour and anger were noted by staff members for several weeks. School achievements and social behaviour improved steadily during the period of inpatient treatment, and, by the time of discharge from hospital the children were functioning socially and educationally at approximately age-appropriate levels.

The parents showed variable degrees of willingness to change. Where change in the child was rapid, and consolidated early, we found that the father made a social effort to be involved with the child and the family, for example, by changing his working hours so that he could be with the family more often. Most parents were surprised when they enjoyed their time with the child during their visits. Although initially reluctant, they joined in activities and games when encouraged and guided by the staff members, who were required to demonstrate to the family how to play together. Once the parents began to enjoy being with their child, their motivation towards change accelerated. Sometimes a grandparent tried to induce the child to return to cross-dressing. Two grandmothers brought in female clothes for their grandsons. These acts caused confrontations between the child and the parents, from which the former retreated.

Outcome at one year

The seven children who completed the treatment programme were seen regularly by their psychiatrist in the outpatient clinic for follow-up. One year after discharge from hospital, all the children were recorded as mixing well with others at school and clinically were observed to be happy. School reports were generally good. The children appeared to have maintained the age-appropriate social skills that had been achieved during their inpatient stay and had continued to mature appropriately. Their self-confidence and sense of mastery, which had been developed while at the hospital, had been maintained in spite of the usual vicissitudes of school and social life.

In one case (Case 1) recurrence of cross-gender behaviour occurred. This child began cross-dressing several months after his discharge from hospital. His father had returned to working excessively long hours and was again absent from home for long periods. The child was readmitted to hospital for two weeks. His father indicated that he was not coping with the marriage. Family discussions followed, changes were made, and the cross-dressing ceased.

Postpubertal status

At the time of the preparation of this report (December 1986), the seven children who completed the therapeutic programme successfully were then aged as follows: one child was 21 years old; two children were 19 years old; one child was 18 years old; one child was 16 years old; one child was 15 years old; and one child was 13 years old. The mean time that had elapsed since admission to hospital was eight years (range, six to 11 years). Contact had been maintained with all the families by the psychiatrist or through the hospital. One family had moved interstate and recent contact had been by telephone. Information was obtained at unstructured interviews with the child and
the parents, and from the examination of school reports.

One young man stereotyped himself when he was 17 years old because he was "mixed up" about his sexuality. He had been actively homosexual since the age of 14 years. However, he did not believe that he was truly homosexual, but explained that he believed he had been "programmed into homosexuality by his mother". He wanted to explore his confusion and fears about women before trying to relate sexually to them. He already had a highly successful career in women's fashions. By the age of 19 years he considered himself to be heterosexual, but had not yet had a sexual relationship with a woman.

None of the other children, now adolescents, expressed homosexual feelings, was transvestite, or transsexual. All had performed reasonably well at school and had reasonable relationships with children of both sexes. All had maintained a sense of well-being. The mother of Patient 5 reported that her son had cross-dressed for a few weeks" at the onset of his puberty. Some subtle pathological influences persisted. One boy (Case 1), aged 15 years, told us that his mother had recently asked him to buy tickets for them to attend a transvestite night club show, a request, he said, which made him feel "very apprehensive".

Discussion
This report can be criticized on a number of levels. The sample is small, and selected by referral to the government service. Because the referrals occurred over a five-year period, and because the follow-up time extends to 11 years, the report provides retrospective descriptions and is not the product of experimental design. Such criticisms could equally be applied to the previous studies of treatment and outcome that have been reported in the literature and are summarized by Zucker. With this qualification, the approach that is described in this report appeared successful in allowing most of the children to overcome their initial unhappiness and to mature in a satisfying manner as human beings at ease with themselves and their sexuality. The treatment brought about an immediate improvement in self-esteem and social functioning, and these aspects appear to have been sustained over many years. Of course, in the later semistructured follow-up interviews, the patients may have been overwilling to oblige their doctors by emphasizing their well-being, or may have been hesitant to communicate desires which they thought might be treated as abnormal. The erotic desires of the seven children who completed the programme, when "grown up", were not known in four cases, two boys (Cases 1 and 8) were heterosexual, and one boy (Case 7) was "mixed". However, all the children were reasonably good at social levels of functioning.

The type of disorders in the family interactions that are reported here, especially the symbiotic relationship with the parent of the opposite sex, are consistent with those that are remembered by gender-disordered adults. While such consistency emphasizes the importance of intrafamilial social learning in the development of gender identity, caution is needed in the extrapolation of clinical findings from small samples of gender-disordered children in an attempt to understand normal psychosexual development. It was surprising how quickly the cross-gender behaviours of the children in our sample ceased once they were away from the home environment. Thereafter, age and sex-appropriate gender behaviours were exhibited by the children. Such a sudden transition suggests that gender behaviours are environmentally developed to a degree that has not been recognized previously. Alternatively, the processes that influence the development of aberrant gender behaviour may not be the same as those that underlie normal psychosexual development.

The descriptions of disordered family relationships may illuminate the aetiology of the disordered behaviours, but normal psychosexual development may, for instance, be more influenced by biological factors. Such distinctions may be important, since others have emphasized the contribution of biological or constitutional factors to the development of gender disorders. For example, on the basis of a comparative study between parents of effeminate and non-effeminate boys, which failed to show distinctions between their attitudes and relationships, Zucker concluded that "the effeminate behaviour was inherent in the boys themselves". This conclusion seems contrary to my findings.

An overemphasis on a biological model of gender disorder may also lead to therapeutic pessimism. Some of the parents of the subjects of this report had been advised by other professional persons that there was "no hope", or that their child would grow up "transsexual" or "homosexual" or, in one instance, that the child "would have to go to New York to have a sex-change operation". Such attitudes, which emphasize cross-gender behaviour as the central or only problem, or which are rigidly deterministic, serve only to deny the child's unhappiness and the desire of the parents to change. Advice that is based on such attitudes fails to provide options to make the lives of the parents and the children more enjoyable. Inpatient treatment is one such option.

Conclusions
The treatment of cross-gender behaviour by means of inpatient therapy seems effective. Long-term clinical evaluations suggest that initial changes can be maintained. These results may correct some previous pessimistic views about outcome. However, evidence about the systematic and objective outcome that would enable one to be more positive about the results is lacking, and the sample is a small one. Further evaluation of therapeutic methods in this area with untreated control families is needed. Such studies will need to be performed in major population centres, since the number of children who meet the diagnostic criteria is relatively few.

Nevertheless, the emphasis on the familial and social context of the disorders that is provided in this report should counteract undue emphasis on the aberrant behaviours themselves. The cross-gender behaviours seemed relatively superficial manifestations of disordered personal interactions and an inadequate repertoire of social skills on the part of both parents and child. Focus on the underlying mechanisms may reveal a great deal about these families, but may not necessarily illuminate the processes of normal psychosexual development.

Acknowledgements
Thanks are due to the staff members of Stuubs Terrace Hospital and of Graylands Primary School and to Dr Ross Kirkman, who helped with the data. I am especially grateful to Dr Ann Whitehead and the late Dr William E. Robinson for kindly allowing me access to their patients, clinical reports and discussions, and to the families and young persons who were good enough to cooperate with us.

References
17. Machover K. Personality projection in the drawing
Barmah Forest virus infections in humans in New South Wales

Royle A. Hawkes, Clement R. Boughton, Helen M. Naim, Barrytt A. Myrick and Lauretta G. Ramsay

ABSTRACT Antibodies to Barmah Forest virus, a member of the alphavirus group, which was first isolated in 1974, have been found to be widespread in humans in New South Wales. Antibody studies showed a higher prevalence in the northern coastal zones of the State, and lower rates in individuals who were living in all other biophysical zones. Antibody rates were significantly higher in male than in female subjects. The pathogenicity of the Barmah Forest virus is at present not known.

A rboviruses are a biologically defined group of viruses which are characterized by the ability to multiply both in arthropods and in vertebrates. More than 60 such agents have been isolated in Australia and in vertebrates. More than 60 such agents have been isolated in Australia. Australian encephalitis (synonym, Murray Valley encephalitis), Kunjin, Ross River and Sindbis viruses and, more recently, Kokobera virus have been shown to cause illness in humans. However, the pathogenicity of the remaining viruses has not been determined.

To determine whether an arbovirus causes illness it is necessary first to show that it can infect humans. A large group of sera, which were collected from NSW blood donors, has already been tested for antibodies to viruses that belong to two families within the arbovirus group, namely, Flaviviridae and the alphavirus genus of Togaviridae. Some of these viruses were shown not to infect humans in New South Wales; others were seen to be likely to cause infections in humans.

Barmah Forest virus was shown in 1984 to be a member of the alphavirus genus. It was recovered originally from Culex annulirostris mosquitoes that were collected in the Barmah Forest of northern Victoria during the summer of 1974, and about the same time from C. annulirostris and Aedes normanensis in Queensland. It has been isolated more recently in the Northern Territory from C. annulirostris and the midge Culicoides marksi, from Aedes bancroftianus in the Barmah Forest, and from Aedes vigilax, Coquillettidia linealis and a pool of Coq. linealis, Coquillettidia modesta and Culex annulirostris mosquitoes that were collected on the south coast of New South Wales. Antibodies to the virus have been found in humans who were living in this area. This brief report concerns the prevalence of Barmah Forest antibody in humans who were living in all biophysical zones of New South Wales. It is an extension of our previous studies.

Materials and methods

Barmah Forest virus BHF193 (sucking mouse brain passage 3, Vero cell culture passage 4, BHK-21 passage 1), Sindbis virus MRM 39 (sucking mouse brain passage 8, BHK-21 passage 1), and Ross River virus T48 (sucking mouse brain passage 6, BHK-21 passage 1) strains were used in the study.

Human survey population

To determine whether a given virus infects humans, it is our policy to select from our total panel of 17,000 sera, samples from 685 individuals who are thought to be at high risk of arboviral infection. These are persons aged 40 years and over from all areas of the State, who are seropositive for alpha and/or flavivirus antibodies. The probability that a donor will be seropositive to an arbovirus is increased if the subject is 40 years of age or older and seropositive for another arbovirus (unpublished observations). If this highly selected sample indicates that a particular virus does infect humans, a larger sample of "standard population" is then tested to determine age- and sex-specific antibody prevalence rates in all biophysical zones of the State (Figure). It comprises roughly equal numbers of subjects from each subzone, who are apportioned into groups with equal numbers (about 30) of male and female persons in the age groups 0-19 years, 20-39 years and 40 years and over.

The original 17,000 donors were selected on the basis of at least three years' residence in a particular locality to exclude transient residents in whom arbovirus antibody patterns may not reflect those of that locality. However, as antibodies may persist for many years and as arboviral infection may be acquired during travel or stay in other areas, the regional patterns that are obtained need to be interpreted with these points in mind.

Serological tests

Haemagglutination-inhibition (HI) test. Barmah Forest viral haemagglutinin for Barmah Forest virus was prepared from infected Vero cell cultures by means of a modification of a previously described method. Infected cell culture fluid was precipitated with polyethylene glycol (PEG 6000) and resuspended in a small volume of borate saline buffer (pH 9). For Sindbis and Ross River viruses, haemagglutinin antigens were prepared from suckling mouse brain, with sucrose-acetone extraction procedures as described previously. Hamagglutination-inhibition tests with Barmah Forest, Ross River and Sindbis antigens were performed with acetone-extracted, goose erythrocyte-absorbed sera.

Microneutralization tests. A previously described micro method was used in which heat-inactivated sera that were diluted one in five were allowed to react with logarithmic dilutions of virus (Barmah Forest, Ross River, Sindbis) and residual virus was assayed by cytopathic effect in Vero cells. Neutralizing antibody was quantitated by the log neutralization index.

Enzyme-linked immunosorbent assay (ELISA)
The ELISA method was based on that of Frazier and Shippe, and was developed for Barmah Forest, Sindbis and Ross River viruses. Infected Vero cell culture fluids, which had been diluted in carbonate buffer (pH 9.6) to a previously determined optimal dilution, were coated on to the solid phase (Immuno Removawell strips; Dynatech, Virginia, USA) overnight at room temperature. Test sera which were diluted one in 100 in PBS-Tween 20 diluent, peroxidase-conjugated antihuman IgG (gamma chain) (Dako; Denmark) which had been prepared in rabbits and was used at a dilution of one in 1000, and an o-phenylenediamine substrate solution (OPD; ICN Pharmaceuticals, New York, USA) were added in sequence, with thorough washing between each step. After the development of colour for 20 minutes the reaction was stopped with 9 N sulphuric acid and plates were read at 492 nm on a Titertek Multiscan ELISA plate reader.

Viral-specific antibody activity was taken as being present in a serum sample when the optical density was greater than the mean plus three times the standard deviation of the readings that were given by sera from a standard sample of six antibody-seronegative donors.

Results

Preliminary studies of high-risk population sample

By means of the HI test, 685 sera were tested...