# Follow-up of a suspected excess of brain tumours among Namibian children

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**To the Editor:** The aim of this follow-up study was to further investigate a suggested excess of childhood brain tumours (CBT) among Herero children in Namibia from 1983 to 1988.[1] Incidence rates of primary brain tumours among Herero children were found to be 4 times higher than rates among Namibian children in any of the 10 other tribal groups or among children of European origin.

The causes of CBTs remain largely unknown. The only established causes are ionizing radiation and predisposing inherited syndromes.[2] A particularly compelling hypothesis is that exposure during gestation to N-nitroso compounds (NOCs) may lead to the development of CBT. This hypothesis was suggested by experimental work in which 100% production of nervous system (NS) tumours in rat offspring resulted from transplacental exposure to the neurocarcinogen ethylnitrosourea (ENU) or to low levels of the precursor compounds sodium nitrite and ethyl urea added to the food and drinking water of pregnant rats.[3] However, when ascorbate (vitamin C) or α-tocopherol (vitamin E) were also included in the diet, endogenous formation of ENU was blocked and tumours did not occur.[3] This effect was shown to occur in numerous species, from rodents to primates, with the greatest susceptibility during gestation.[4] ENU precursors are readily available in human diets and other common exposures. Drinking water is one possible source of nitrate, which is readily reduced to nitrite in the presence of human saliva. When levels in water are high drinking water can be the major source of this NOC precursor; for example water containing > 50 mg/l nitrate ion can represent 70% or more of total dietary intake.[5]

The first step in this follow-up investigation was to update the CBT incidence survey to include tumours first diagnosed from 1989 to 1997. Before 1989 most Namibian children with cancer were referred to the paediatric oncology unit at Tygerberg Hospital (TBH) in Cape Town.[6] After this date treatment facilities in Namibia were improved, and patients were mostly referred to TBH only for radiotherapy.[6] Copies of reports of brain tumours occurring among Namibian children from 1989 to 1997 were requested from the central tumour registry of Namibia and were reviewed by the investigators at TBH in Cape Town. Based on the child's surname, Herero children were identified from among all CBT cases. Further verification of Herero ethnicity was obtained by examining referral patterns from the local clinic where the child first presented to regional medical facilities and finally to the central paediatric oncology ward in Windhoek. The initial incidence survey found a total of 28 cases of CBT among Namibian children in the 6 years from 1983 to 1988; 6 of these were among Herero children, who comprise about 10% of the paediatric population. Only 1 incident of CBT occurred among Herero children during the 9 years from 1989 to 1997 out of a total of 24 in all Namibia. Had the excess seen in 1983 - 1988 continued, 8 new cases among Herero children would have been expected for the period 1989 - 1997. In fact the number of new CBT cases diagnosed per year also declined among non-Herero Namibian children from 3.67/year during 1983 - 1988 to 2.56/year during 1989 - 1997.

Secondly, a field survey was also conducted and samples of drinking water were collected from sources used during pregnancy by mothers of Herero children who had brain tumours diagnosed from 1983 to 1988. Samples were collected with the help of public health nurses for dipstick measurements of nitrite and nitrate levels along with 'convenience control' samples from locations the investigator visited (Ministry of Health and Social Services (MHSS) offices) or stayed at during this survey. These dipsticks (Merck, Darmstadt) were read on location and were similar to those used previously in an international study of CBT and content of maternal drinking water.[5] Dipstick findings were confirmed in more detailed laboratory analyses performed in the USA. At each case and control location 2 samples were used to test on site for nitrites and nitrates, and 2 additional samples were stored on dry ice and shipped on dry ice within 24 hours from Windhoek to Minneapolis, Minnesota, USA for analysis. Labels used on each sample ensured that the laboratory was blinded as to case or control status of the samples.

Dipstick measurements were done for 12 water samples (Table I). The relevant water source was only available for 3 of the 6 CBT cases diagnosed from 1983 to 1988, although in the case of 1 of these mothers we were only able to sample from the municipal supply where the woman now lives. She had not previously told the public health nurse that during the

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**Table I. Dipstick measurements of nitrates and nitrates from water samples from locations in Hereroland and central Namibia in 2000**

| Case No. | Water samples* | Nitrate (mg/l) | Nitrite (mg/l) (ion)† | Nitrite (mg/l) (ion)‡ | Comments |
|---|---|---|---|---|---|
| 1 | ca/ru | 250 | --- | --- | Mother and child deceased. Father took us to the small settlement where the mother had lived, in order to take samples |
| 2 | ca/pu | 0 | --- | 0 | Water sampled from municipal supply where the mother now lives. During the pregnancy she had lived on a rural Herero reserve. There was insufficient time to visit it and no one to show us how to get there |
| 3 | ca/ru | 100 | --- | 2 | Well water delivered by truck to the farm where the mother had worked |
| 4 | co/ru | 10 | --- | 0 | 50 m well on guest farm |
| 5 | co/pu | 0 | --- | 0 | National park guest cabin§ |
| 6 | co/ru | 50 | --- | 0 | Regional well supplying small clinic |
| 7 | co/pu | 25 | --- | 0 | Municipal water in MHSS office |
| 8 | co/pu | 0 | --- | 0 | Municipal water in MHSS office |
| 9 | co/pu | 0 | --- | 0 | National park guest cabin§ |
| 10 | co/pu | 25 | --- | 0 | National park guest cabin§ |
| 11 | co/pu | 10 | --- | 2 | National park guest cabin§ |
| 12 | co/pu | 10 | --- | 0 | Municipal supply to residential suburb of Windhoek |
| 13 | co/pu | 0 | --- | 0 | Municipal supply to residential suburb of Cape Town |

*Status: ca/co = case or control water sample; ru/pu = water sampled from a private rural or public source, usually in a town on municipal or other public water supply. Private rural locations got water from boreholes.
†Nitrite level from dipstick with two strips, one for nitrites and one for nitrates.
‡Nitrite level from dipstick with single strip to measure nitrites only.
§Each national park location was distinct. Included are samples from guest cabins in Waterberg, Daan Viljoen, and two locations in Etosha - Namutoni and Okaukuejo.
MHSS = Ministry of Health and Social Services (water was sampled from the MHSS regional offices in Otjwarango and Gobabis).

relevant pregnancy she had lived mostly on a rural Herero reserve; there was insufficient time to visit the remote location and no one available to show the way. Two families could not be traced. During her pregnancy another mother had lived in a building previously receiving water from a private source, but the new building in that location was now on the municipal water supply. Two samples had high nitrate levels (250 and 100 mg/l (ion) respectively); both were from rural water supplies close to where case mothers had lived during their pregnancies. Four samples of municipal or national park water had no detectable nitrates, 3 had levels of only 10 mg/l, 2 had levels of 25 mg/l, and a rural clinic had a level of 50 mg/l.

Laboratory measurements using various methods of assaying nitrate levels conducted in the USA for 6 highest priority samples (numbers 1 - 6 listed in Table I) in general confirmed dipstick findings. Three of the 4 samples from boreholes/wells (2 case samples and the clinic sample) from rural water supplies showed nitrate levels at (clinic) or above (2 cases) the World Health Organization (WHO) recommended limit of 50 mg/l; all 3 showed these high levels on 2 or more of the assays used. None of the samples from municipal supplies or national parks had levels above 25 mg/l on any assay.

Drinking water is a potential source of nitrates, which are readily reduced to nitrites in the presence of saliva. When levels in water are high, as they were in some wells used by the Herero population, it can be the major source of this NOC precursor,[4] and if the diet includes relatively low levels of nitrosation inhibitors such as vitamins C or E, endogenous formation of carcinogenic NOC is likely to occur.[2] Nonetheless, the potential carcinogenic effect of this exposure is unclear, but an increased risk of CBT associated with nitrates in drinking water is at least plausible.[4]

While our results related to the dipstick measurements are not affected by recall bias or other limitations of self-reported data, they do have some limitations. Measurements were conducted several years after women's pregnancies; even if the residences remained the same, water quality and content may have changed. Other limitations are the semi-quantitative nature of the dipsticks, the necessary subjectivity in interpretation of results by the rater, and the possibility that despite the attempt to adhere to the manufacturer's instructions, some interferences and inaccuracies may have occurred in their use.

A major limitation of our study is the very small number of cases; both the number of incident cases among Herero and other children in Namibia from 1989 to 1997 and the number of water samples tested were too few to justify testing for statistical significance. In addition questions remain relating to the different methods of case ascertainment during the two time periods. Nonetheless, a fourfold excess of a serious rare disease such as CBT represents an opportunity that warrants investigation.

According to the WHO and European Union maximum admissible concentration standards, the maximum allowable

nitrate level for drinking water is 50 mg/l nitrate ion. In most countries drinking water from private sources such as boreholes is less closely regulated than water from public supplies. Homes with water from local boreholes are also more likely to be located in agricultural areas with the possibility of exposure to various infectious and chemical agents from sources other than drinking water; information was not collected on such possible exposures from farm animals or agricultural chemicals. Furthermore, drinking water with high nitrate levels frequently also includes other contaminants, such as pesticides and metals,[5] which were not measured in this study.

In summary, our study showed that the previously observed apparent excess incidence of primary brain tumours among Herero children from 1983 to 1988 did not continue after 1989. Further, our study demonstrated that the extraordinary helpfulness of the public health workers in Namibia made follow up of families of these children possible even after 15 or more years since last contact Water sampling suggests that nitrate levels may be high in boreholes in some rural areas of Hereroland; however, water from municipal supplies in Namibia appears to contain low levels.

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Before its initiation, this investigation was approved by the Ethical and Scientific Review Board of the Faculty of Health Sciences at Stellenbosch University in the Western Cape and by the Namibian Ministry of Health and Social Services in Windhoek.

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Analyses of water samples were conducted in the Aqueous Geochemistry Laboratory, Department of Geology and Geophysics, University of Minnesota on a Dionex DX-500 ion chromatograph, with a CD 20 detector, a GP 40 pump, and 2 AS4A anion exchange columns eluted with carbonate/bicarbonate at a flow rate of 2.1 ml/minute.

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