Clinical signs of trachoma and laboratory evidence of ocular *Chlamydia trachomatis* infection in a remote Queensland community: a serial cross-sectional study

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The known: Trachoma was formerly endemic in northwest Queensland.

The new: Three annual surveys in a remote Queensland community in which trachoma had been endemic found clinical signs consistent with follicular trachoma in 7% of examinations of children aged 5–9 years, beyond the 5% threshold for which community-wide azithromycin treatment is recommended. However, additional clinical and laboratory assessments suggested that childhood ocular *Chlamydia trachomatis* infections were unlikely.

The implications: Simplified clinical grading can overestimate the burden of trachoma in low prevalence settings, leading to unnecessary antibiotic treatment and further screening. Assessing progress to trachoma elimination in Australia and elsewhere should therefore incorporate laboratory testing.

Abstract

Objectives: To compare the findings of standard clinical assessments and of complementary clinical and laboratory methods for determining whether community-wide treatment for trachoma is warranted in a remote Queensland community.

Design: Three cross-sectional screening surveys, 2019–2021, complemented by laboratory pathology testing.

Setting: Small community in northwest Queensland with geographic and cultural ties to Northern Territory communities where trachoma persists.

Participants: Children aged 1–14 years; opportunistic screening of people aged 15 years or more.

Main outcome measures: Prevalence of clinical signs of trachoma, *Chlamydia trachomatis* infection, ocular non-chlamydial infections, and seropositivity for antibodies to the *C. trachomatis* Pgp3 protein.

Results: During the three surveys, 73 examinations of 58 children aged 1–4 years, 309 of 171 aged 5–9 years, and 142 of 105 aged 10–14 years for trachoma were undertaken, as were 171 examinations of 164 people aged 15 years or more; 691 of 695 examinations were of Aboriginal or Torres Strait Islander people (99%), 337 were of girls or young women (48%). Clinical signs consistent with trachomatous inflammation–follicular were identified in 5–9-year-old children 23 times (7%), including in eleven with non-chlamydial infections and one with a *C. trachomatis* infection. One child (10–14 years) met the criteria for trachomatous scarring. Two of 272 conjunctival swab samples (all ages) were polymerase chain reaction-positive for *C. trachomatis* (0.7%). Two of 147 people aged 15 years or more examined in 2019 had trichiasis, both aged 40 years or more. Seven of 53 children aged 1–9 years in 2019 and seven of 103 in 2021 were seropositive for anti-Pgp3 antibodies.

Conclusions: Despite the prevalence of clinical signs consistent with trachomatous inflammation–follicular among 5–9-year-old children exceeding the 5% threshold for community-wide treatment, laboratory testing indicated that childhood exposure to ocular *C. trachomatis* is rare in this community. Laboratory testing should be integrated into Australian trachoma guidelines.

Trachoma is the most frequent infectious cause of blindness. In 1996, the World Health Organization (WHO) aimed to eliminate it as a public health problem by 2020, but in its recent road map for neglected tropical diseases revised the target date to 2030.[1] To date, fifteen countries have eliminated trachoma as a public health problem.[2]

Australia is the only high income country in which endemic trachoma persists, primarily in remote Aboriginal and Torres Strait Islander communities.[3] Trachoma is strongly associated with poverty,[4] and its persistence in First Nations communities is inextricably linked with social inequalities, the ongoing legacy of colonisation. The number of Australian communities at risk of trachoma has declined since 2009, but in many the prevalence of trachomatous inflammation–follicular remains high.[3]

Active trachoma — trachomatous inflammation–follicular (TF) or trachomatous inflammation–intense (TI) — is a conjunctival inflammatory response (upper tarsal follicular conjunctivitis) to infection with ocular strains of *Chlamydia trachomatis*.[4] Repeated episodes during childhood cause conjunctival scarring, which can lead to trachomatous trichiasis, corneal opacity, and, ultimately, blindness.[4,5] Mathematical modelling suggests that more than 150 ocular *C. trachomatis* infections are required before trachomatous trichiasis develops, and that the earlier and more frequent the infections, the earlier in life trichiasis develops.[5]

In screening programs, trachoma is diagnosed using the WHO simplified five sign grading system.[6] The prevalence of active trachoma among 5–9-year-old children guides public health interventions; if it is 5% or more, the community is classified as being at risk of trachoma, and Australian guidelines recommend three rounds of community-wide azithromycin treatment at 12-month intervals.[6]

Simplified clinical grading has been useful in Australia and overseas for tracking progress towards eliminating trachoma. However, the specificity and positive predictive value of TF for estimating the prevalence of *C. trachomatis* infections may be poor for populations in which treatment has been undertaken or the prevalence of trachoma is low.[7,8] Findings

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from the Torres Strait Islands and across the Pacific suggest that simplified clinical grading can lead to overestimating infection prevalence.[9-13] As the predictive value of simple clinical assessment declines, other tools may be more useful for estimating trachoma prevalence, including assessment of conjunctival scarring and anti-Pgp3 seropositivity, each recognised as persistent markers of previous active trachoma and *C. trachomatis* infection.[14,15]

We have previously reported that simplified grading caused overestimation of the prevalence of trachoma in a Torres Strait Island community.[9] We have therefore supplemented data from surveys in northwest Queensland recommended by the National Trachoma Surveillance and Control Reference Group by collecting additional information to determine whether community-wide treatment and further screening rounds are required.

Methods

We undertook annual, cross-sectional trachoma prevalence surveys during 2019–2021 (October 2019, February 2020, February 2021) in a single northwest Queensland community (population: under 1500), selected because of geographic and cultural ties with Northern Territory communities where trachoma persists. Consistent with Australian guidelines, we aimed to screen at least 85% of 5–9-year-old children present and resident in the community.[6] To provide a more comprehensive epidemiological picture of trachoma, we also promoted screening of children aged 1–4 or 10–14 years, and opportunistically screened people aged 15 years or more.

In 2019, we also collected dried blood spots from people (all ages) to assess for the *C. trachomatis* plasmid-encoded Pgp3 protein, and conjunctival swabs for polymerase chain reaction (PCR) testing for *C. trachomatis*. Three months after the 2021 prevalence survey, we again collected dried blood spots for anti-Pgp3 serology.

The approach we took for achieving community engagement and approval is described in detail in the Supporting Information. Our report conforms with STrengthening the Reporting of OBservational studies in Epidemiology (STROBE) guidelines.[16]

Clinical examination

Participants were assessed for the five signs of trachoma (WHO simplified grading criteria[6]) by an ophthalmologist using binocular magnifying loupes (2.5× magnification) and a torch for illumination.[6] Eyes were also assessed for Herbert's pits and corneal pannus, signs considered by many experts to be enduring cicatricial markers of current or previous active trachoma.[17,18] Each child screened for trachoma was assessed for "clean face", defined as the absence of nasal and ocular discharge.[6] The ophthalmologist and assisting health worker cleaned their hands with alcohol-based hand gel after each examination.

Specimen collection

Immediately after clinical examination, conjunctival specimens were collected with cotton swabs from both eyes of participants with any follicles in the upper tarsal conjunctiva of either eye (in 2019: from all serosurvey participants), and immediately placed into Cobas PCR media tubes (4.3 mL) for *C. trachomatis* PCR assessment. Conjunctival specimens were collected in the same manner for bacterial culture and placed into sterile tubes of swab medium (Amies transport gel) (further details: Supporting Information).

Dried blood spots were collected in 2019 and 2021 by finger prick (up to five spots; about 50 μL whole blood per spot) onto Whatman 903 protein saver cards (ThermoFischer Scientific Australia).

Laboratory testing

Conjunctival swabs underwent dual mode PCR testing for *C. trachomatis*/*Neisseria gonorrhoeae* (Cobas 6800, Roche Diagnostics). Pathology Queensland laboratories (Mount Isa; more than 300 km from the community) prepared cultures of bacteria that cause follicular conjunctivitis, as well as pure growths of Gram-negative enteric bacteria. Anti-*C. trachomatis* antibody (anti-Pgp3) in dried blood spots was detected by enzyme-linked immunosorbent assay (ELISA)[19] (further details: Supporting Information).

Data recording and analysis

We assumed TF prevalence among children aged 5–9 years was 10%; given the community population number, we needed to recruit 110–117 children to estimate the community proportion with an absolute precision (95% confidence interval, CI) of two percentage points.

Demographic and examination survey data were recorded for each participant. Data were entered into Excel (Microsoft), entry integrity checked, and descriptive epidemiological analyses performed. Data are summarised as frequencies and proportions. We assessed seroprevalence only for children aged 1–9 years. Seroconversion rates (with 95% CIs) were calculated using a generalised linear model with binomial family and identity link function. The association between age and anti-Pgp3 seropositivity was investigated in a generalised linear model with binomial family and logit link function. Regression models were weighted to reflect the number of resident children in each age group year. Analyses were conducted in Stata 14.

Ethics approval

The Townsville Hospital and Health Service Human Research Ethics Committee (HREC) provided an ethics waiver for publishing the survey findings (EX/2021/QTHS/77180). The laboratory component of our study was approved by the Townsville Hospital and Health Service HREC (HREC/2019/QTHS/54887) and the University of Queensland HREC (2019002412/HREC/2019/QTHS/54887). The Aboriginal Shire Council provided community approval for our study. Written consent was provided by all participants or, for children under 18 years of age, by parents or guardians.

Results

Across the three clinical surveys, we undertook 73 examinations of 58 children aged 1–4 years, 309 of 171 children aged 5–9 years, and 142 of 105 children aged 10–14 years for trachoma, as well as 171 examinations of 164 people aged 15 years or more. A total of 691 of 695 examinations (all ages) were of Aboriginal or Torres Strait Islander people (99%), and 337 examinations were of girls or young women (48%) (Box 1). The participation rates for 5–9-year-old children exceeded 85% of the age-specific resident population in 2020 and 2021, but not the 2019 survey (Box 2).

1 Characteristics of annual clinical survey participants from a northwest Queensland community, 2019–2021

Characteristic	Survey year		
	2019*	2020	2021
People screened	281	210	204
Aboriginal or Torres Strait Islander people	277 (99%)	210 (100%)	204 (100%)
Age (years), median (range)	19 (1–89)	8 (1–40)	9 (2–76)
Age group (years)			
1–4	20 (7%)	33 (16%)	20 (10%)
5–9	70 (25%)	126 (60%)	113 (55%)
10–14	44 (16%)	46 (22%)	52 (26%)
15 or older	147 (52%)	5 (2%)	19 (9%)
Sex			
Female	147 (52%)	98 (47%)	92 (45%)
Male	134 (48%)	112 (53%)	112 (55%)

* In 2019, as we had not previously performed a serosurvey in this community, we encouraged participation by people from all age groups; in the later surveys, we sought better representation of younger people, particularly 5-9-year-old children. ◆

Ocular swabs for *C. trachomatis*/*N. gonorrhoeae* PCR testing were collected from 272 participants (78 with and 194 without follicles on the upper tarsal conjunctiva). Swabs for bacterial culture testing were collected from 79 participants (78 with follicles and one with non-trachomatous eye symptoms). A total of 448 dried blood spot samples were collected during the surveys conducted in 2019 (221 samples) and 2021 (227 samples) (Box 2).

Examination findings

Across the three surveys, TF was identified 23 times during 309 examinations of children aged 5–9 years (7%): six of 70 in 2019 (9%), seven of 126 in 2020 (6%), and ten of 113 in 2021 (9%). TF was identified in three children in two surveys, and in one child in three surveys. Among children aged 1–4 years, TF was identified in two of 20 in 2019 (10%), none of 33 in 2020, and three of 20 in 2021 (15%) (Box 2).

In 2019, one child (5–9 years) had bilateral TI (and bilateral TF) and one child (10–14 years) met the criteria for trachomatous scarring. No other child had TI or trachomatous scarring, nor were trichiasis, Herbert's pits, or corneal pannus detected in any child (1–14 years). Eleven of 171 clinical examinations of participants aged 15 years or more identified trachomatous scarring (6%).

In 2019, 147 of 852 residents aged 15 years or more (17%) were screened for trichiasis. Two cases were identified (both in people aged 40 years or more): one was associated with unilateral trachomatous scarring, the other was bilateral, but eversion of the lids and further examination was not possible because of eyelash epilation. Both people were referred for further investigation and management. Three participants had corneal opacity (2%) and 18 had Herbert's pits (12%), none of whom had trichiasis. Apart from one case of Herbert's pits in a person aged 15–39 years, all cases of corneal opacity and Herbert's pits were in people aged 40 years or more. No evidence of corneal pannus was detected in an adult.

Clean faces were noted for 48 of 70 5–9-year-old children in 2019 (69%), 105 of 126 in 2020 (83%), and 82 of 113 in 2021 (73%).

Ocular *C. trachomatis* infection

Two of 272 collected samples (0.7%) were PCR-positive for *C. trachomatis*, both in 2019 (Box 2). Both participants, members of the same family, had bilateral ocular *C. trachomatis* infections; one (5–9 years) had bilateral TF and TI, the other (10–14 years) had no clinical signs of trachoma. No participants were PCR-positive for *N. gonorrhoeae*. No other bacterial organisms were isolated from the eyes of these two children.

Bacterial cultures

Bacteria could be cultured from 28 of 79 conjunctival samples (35%), including eleven of 23 samples from 5–9-year-old children with TF (48%), most frequently β-lactamase-negative *Haemophilus influenzae* (four cases) and methicillin-resistant *Staphylococcus aureus* (four cases). In 2019, a bacterial swab was collected from the right eye of one child (10–14 years old) for a clinical indication other than trachoma; nothing grew within 48 hours (Supporting Information, table).

Anti-Pgp3 serology

Among children aged 1–9 years, seven of 53 in 2019 (13%) and seven of 103 in 2021 (7%) were seropositive for anti-Pgp3 antibodies (Box 2); in 2021, seropositivity by individual year of age ranged from 0 (1, 2, 4, or 7 years of age) to 33% (5 years, two of six children). The estimated seroconversion rate was –0.7% (95% CI, –3.0% to 1.6%) per year, and the point estimate for the odds of being seropositive declined by 9.6% (95% CI, –33% to +21%) per year of age.

Antibody data were available for eight of the 23 children aged 1–9 years with TF identified in any survey; one was seropositive for anti-Pgp3 antibodies (13%). The only 5–9-year-old child with TI was seropositive; the child in the 10–14-year age group who met the criteria for trachomatous scarring was seronegative. Both children PCR-positive for *C. trachomatis* were seropositive for anti-Pgp3 antibodies.

Among the participants aged 15 years or more for whom we had antibody data, 114 of 144 were seropositive for anti-Pgp3

2 Trachoma screening and laboratory pathology assessment of participants from a northwest Queensland community in annual cross-sectional surveys, 2019–2021

Participant characteristic	Survey year and age group (years)											
	2019				2020				2021			
	1–4	5–9	10–14	≥ 15	1–4	5–9	10–14	≥ 15	1–4	5–9	10–14	≥ 15
Estimated resident population	58	125	67	852	99	135	149	852	81	128	103	863
Examined for trachoma	20 (34%)	70 (56%)	44 (66%)	147 (17%)	33 (33%)	126 (93%)	46 (31%)	5 (1%)	20 (25%)	113 (88%)	52 (50%)	19* (2%)
Follicular trachomatous inflammation†	2 [10%]	6 [9%]	1 [2%]	1 [0.7%]	0	7 [6%]	0	0	3 [15%]	10 [9%]	2 [4%]	0
PCR swab collected	2	6	1	1	—	7	—	—	3	10	2	—
C. trachomatis-positive	0	1	0	0	—	0	—	—	0	0	0	—
1–4 upper eyelid follicles	1 [5%]	8 [11%]	4 [9%]	3 [2%]	0	4 [3%]	0	0	2 [10%]	19 [17%]	5 [10%]	0
PCR swab collected	1	8	4	3	—	4	—	—	2	19	5	—
C. trachomatis-positive	0	0	1	0	—	0	—	—	0	0	0	—
No upper eyelid follicles	17 [85%]	56 [80%]	39 [89%]	143 [97%]	33 [100%]	115 [91%]	46 [100%]	5 [100%]	15 [75%]	84 [74%]	45 [86%]	19 [100%]
PCR swab collected	13	25	20	136	—	—	—	—	—	—	—	—
C. trachomatis-positive	0	0	0	0	—	—	—	—	—	—	—	—
Clean face	9 [45%]	48 [69%]	44 [100%]	147 [100%]	16 [48%]	105 [83%]	43 [93%]	5 [100%]	7 [35%]	82 [73%]	50 [96%]	4* [100%]
Dried blood spot collected for serology	19 (33%)	34 (27%)	24 (36%)	144 (17%)	—	—	—	—	33 (41%)	70 (55%)	62 (60%)	62 (7%)
Anti-Pgp3-seropositive	1 [5%]	6 [18%]	6 [25%]	114 [79%]	—	—	—	—	2 [6%]	5 [7%]	16 [26%]	42 [68%]

PCR = polymerase chain reaction testing; Pgp3 = *Chlamydia trachomatis* antigen Pgp3. * 15 people were examined by a registered nurse from the trachoma program for trichiasis only. † Presence of five or more follicles on the upper tarsal conjunctiva of at least one eye. ◆

antibodies in 2019 (79%) and 42 of 62 in 2021 (68%). Both adults with trachomatous trichiasis were seropositive, as were eight of eleven with trachomatous scarring (73%).

Discussion

In one remote northwest Queensland community, TF was identified 23 times during 309 examinations of 171 children aged 5–9 years during 2019–2021 (7%), and the proportion exceeded 5% in each of the three surveys. According to Australian guidelines, three rounds of community-wide treatment over 24 months would therefore be required.[6] However, our clinical and laboratory assessments provide important further details about trachoma epidemiology in this community.

Firstly, we found little evidence for current ocular *C. trachomatis* infections; across three surveys, only one 5–9-year-old child with TF was *C. trachomatis*-positive. In areas where the prevalence of trachoma is below 10%, it is estimated that as many as 19% of children with active trachoma are PCR-positive for *C. trachomatis*.[20]

Secondly, we found little evidence for early life exposure to *C. trachomatis*. The overall anti-Pgp3 seroprevalence for 1–9-year-old children was 13% in 2019 and 7% in 2021, and the point estimate for the odds of being seropositive declined by 9.6% (95% CI, –33% to +21%) per year of age for each year of age. In Western Pacific communities where trachoma is endemic, anti-Pgp3 seroprevalence in children aged 1–9 years ranges between 35% and 59%, and increases with age.[19,21]

Thirdly, no participant had corneal pannus, and no-one with TF had Herbert's pits. In communities in Guinea Bissau and Taiwan in which trachoma is endemic, 60–80% of people with TF and 20–40% of those without active trachoma have Herbert's pits or pannus.[15,22,23]

Finally, only one 5–9-year-old child had clinical signs consistent with TI. In communities where trachoma is endemic, TI is frequently encountered in young children; in the long term, it is the best predictor of trachomatous scarring in adults.[24] Taken together, these results suggest that childhood ocular

C. trachomatis infections are rare in this community and unlikely to explain most of the TF cases we identified.

In 2019, two of 147 examined people aged 15 years or more (both were at least 40 years old) had trachomatous trichiasis, exceeding the 0.1% threshold set by Australian national guidelines for the presence of endemic trachoma.[6] Finding trachomatous scarring and Herbert's pits in adults was unsurprising, and is consistent with surveys undertaken in 1977 that reported trachoma to be endemic in the Queensland region in which the community is located.[25]

Trachoma is strongly associated with environmental factors such as overcrowded households, and inadequate access to water and sanitation facilities.[26] Housing supply in remote communities is subject to complex interactions between federal, state, and territory authorities, leading to unclear responsibility and accountability for policy, insufficient investment, and inadequate coordinated planning, expenditure, and monitoring.[27] Activities for improving environmental health are funded under a Federation Funding Agreement,[28] but more needs to be done. The low numbers of trichiasis cases in the community we surveyed reminds us of the continuing impact of historical trachoma; continued screening and management of trichiasis in older community members will be required wherever trachoma was once endemic.

Limitations

The major strength of our study was the integration of trachoma screening and laboratory investigations. It was the first Australian study to assess the seroprevalence of anti-Pgp3 antibodies, which we found to be a useful approach. However, we did not have a complete list of community residents, and the highly mobile population — members are often absent from the community for extended periods — made it difficult to estimate the number of residents. Consequently, we could not examine "all" children aged 1–9 years or assess reasons for refusing participation in our study. As our primary focus was public health screening, our laboratory investigations were also focused on children aged 1–9 years. Nevertheless, we also examined 25–33% of children aged 1–4 years for each survey. We could not collect data on individual risk factors, such as history of or exposure to trachoma and environmental risk factors. Collecting specimens from the entire community would have been ideal, but was precluded by resource constraints. We report findings for only a single community, but it had previously been identified as being at risk of trachoma.[25]

Conclusion

In a study that reflected trachoma screening programs in remote Australia, we found that, while it was probably once endemic in the remote Queensland community we surveyed, this is no longer the case. Based on these and similar findings elsewhere in north Queensland, the National Trachoma Surveillance and Control Group endorsed discontinuing routine screening in this community in 2022. Indeed, following our Torres Strait Islands investigation,[9] routine public health screening for trachoma is no longer required in any Queensland community.

Our findings have implications for how trachoma screening is performed, and how progress towards elimination is monitored in both Australia and other countries where clinical signs of trachoma persist despite repeated community-wide antibiotic treatment. Following current Australian guidelines, without the benefit of the data on infections and seropositivity we collected, this community would have undergone an unnecessary cycle of repeated screening and treatment. We have shown the added benefit of detailed clinical examination, PCR testing, and assessment of anti-Pgp3 antibody seroprevalence alongside routine public health surveys. As Australia approaches trachoma elimination, laboratory testing, including PCR testing and serology, should be incorporated into national guidelines.

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Supporting Information

Additional Supporting Information is included with the online version of this article.