HIV infection predominantly affecting children in Sindh, Pakistan, 2019: an outbreak report

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Abstract

Background: In April 2019, an HIV screening camp for all ages was established in response to a report of an unusually large number of paediatric HIV diagnoses in Larkana, Pakistan. A cross-sectional study to understand the clinical profile of children who registered for HIV care was performed.

Methods: Age- and sex-stratified HIV prevalence among individuals screened was estimated. Data on children who registered for HIV care including clinical history, HIV disease stage, Hepatitis B and C status and CD4 count was abstracted from clinical records and analysed using percentages and chi square tests.

Findings: Between April-July 2019, 31,239 individuals underwent HIV testing of whom 930 tested HIV-positive. Of these, 763 (82·0%) were aged <16 years. Estimated HIV prevalence was 3.0% overall; 7.4% (283/3803) in children aged 0-2 years, 5.9% (321/5412) in children aged 3-5 years, and 1·3% (148/11251) in adults aged 16-49 years. Of the 591 children who registered for HIV care, 80.9% (478/591) were ≤5 years, 64·1% (379/591) were male and 53·4% (315/590) had a weight-for-age Z-score <-3. Hepatitis B surface antigen and Hepatitis C antibody positivity was 8·4% (48/574) and 2·6% (15/574) respectively. Of children whose mothers tested for HIV, only 39/371 (10·5%) had HIV-positive mothers. Most children (89·2%, 404/453) reported multiple previous injections and 40/453 (8·8%) reported blood transfusions.

Interpretation: This is an unprecedented HIV outbreak among children in Pakistan: a 54% increase in paediatric HIV diagnoses over the past 13 years. Epidemiological and molecular studies to understand the full extent of the outbreak and its drivers are needed to guide HIV control strategies.

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Background

Pakistan is among the four countries in Asia where the estimated number of new HIV infections has been increasing annually since 1990. In 2017, 150,000 adults and children were estimated to be living with HIV in Pakistan of whom only 3500 (<2·5%) were children aged below 15 years - a 127% increase in numbers of people living with HIV from 2010.[1]

Traditionally, the HIV epidemic in Pakistan has been concentrated among key populations that include persons who inject drugs (PWID), men who have sex with men (MSM) and male, female and transgender sex workers.[2] The HIV epidemic among PWID is well-established with prevalence of over 35% observed among PWID in large cities.[3, 4] HIV prevalence is somewhat lower in the sex worker population nationally, ranging between 1% (female sex workers) to 7% (transgender sex workers),[4] although HIV incidence is projected to rise among all the defined key population groups over the next decade.[5, 6] Reasons include low literacy, low levels of awareness and knowledge on how to prevent HIV infection, lack of adequate prevention services, strong cultural taboos and discrimination towards these often marginalized groups by both state and non-state actors.[7, 8] As a result of limited systematic HIV screening, significant geographical heterogeneity in patterns and trends of HIV sub-epidemics,[5] and varying degrees of social and sexual interaction between key populations and the wider community across Pakistan, there is uncertainty of the extent of HIV transmission to the general population.[6, 9-11] However until now, children have rarely been identified as a population affected and antenatal HIV screening is only offered in 8 Prevention of Parent-to-Child HIV Transmission Centres across the country.

On April 18, 2019, a thalassemia care service that provided blood transfusions informed the paediatric HIV service in Karachi that 46 children with thalassemia who had previously used other services, registering at their new centre in Larkana, a rural district in Sindh province, tested positive for HIV. These children were also screened for Hepatitis B and C and 24/46 (52%) and 3/46 (7%) had Hepatitis B and Hepatitis B/Hepatitis C co-infection respectively.

On the same day, a report that fourteen additional children had been diagnosed with HIV in Ratodero, a town within Larkana district, was broadcast on several news media channels. This led to the unearthing of a much larger HIV outbreak in Ratodero. We review the outbreak response from the government, academia and United Nations (UN) agencies in Larkana, Sindh. We also present demographic data on individuals screened and clinical characteristics of children who registered for care.

Methods

Ratodero *tehsil* (town) is situated within Larkana district in the Sindh province, which is the second most populous of Pakistan's five provinces. Ratodero has a population of 331,584,[12] and 55% of households live below the poverty line.[13]

Following the widespread reporting of newly-diagnosed paediatric HIV cases, the provincial and federal government responded rapidly to the outbreak to create a taskforce that included provincial and district health and administrative authorities, experts from academic institutions and relevant UN agencies. A taskforce conducted visits to private and public sector health facilities and the Extended Programme of Immunisation (EPI) and observed use of unscreened blood products, reuse of needles in administration of antibiotics and fluids and inadequate sharps disposal systems in health facilities but the EPI programme had good standards. These observations led to the hypothesis that HIV was predominantly transmitted through poor infection prevention practices and resulted in the closure by the Sindh Health Care Commission (a regulating body of the Ministry of Health, Sindh) of three blood banks (one private and two public sector) in Larkana and almost 300 clinics run by untrained health care providers in Sindh.[14] A special committee was established to address the lack of infection control in health facilities and blood safety. As part of a community mobilization campaign, formal meetings (autaq) between community leaders, local government and health officials were held and HIV awareness sessions with community health workers including lady health workers, community-based organisations, law enforcement agencies, informal healthcare providers (barbers) and health care providers were conducted.

Free voluntary HIV counselling and testing was offered to all age-groups from the 25th of April 2019 at a screening camp established at the Sindh public sector hospital Tehsil Hospital Ratodero by the Sindh provincial Ministry of Health in response to the outbreak. HIV testing was performed using the National HIV testing guidelines.[15] Initially data on age, sex and residence was collected using paper records, but a biometrics system linked to an electronic database (to prevent repeat testing of individuals) was established from May. For analysis, we assumed that the distribution of age-group and gender was the same among those for whom data was not available as the known distribution in those with complete data, and used these denominators to estimate HIV prevalence by age-group and sex for the whole screened population.

Adults who tested positive were referred to the existing HIV care centre at Civil Hospital, Larkana. Children who tested positive were referred to a new paediatric HIV clinic established by the Sindh AIDS Control Program (SACP) within a week of the outbreak at Shaikh Zayed Childrens Hospital, Larkana. Data on children, defined as those aged below 16 years, who registered for HIV care was extracted from the national electronic HIV database (Management Information System (MIS) for HIV & AIDS). The system is designed to track numbers of patients in HIV care across the country. Each patient registering for HIV care is given a unique identifier and patients who transfer to other centres retain the same identifier. Each attendance is recorded and the system thus enables tracking of patients who are lost-to-follow-up (defined as not having attended for a clinic appointment for 6 months) or transfer to other service providers. At the initial visit, detailed socio-demographic and clinical history and likely source of HIV infection including mother-to-child transmission, history of blood transfusions and multiple injection and cannulations is recorded on a standardised form, and entered into the electronic MIS.

Children who registered for HIV care had a full blood count, CD4 count, Hepatitis B surface antigen and Hepatitis C antibody testing done. Patients with thalassemia also underwent Venereal Disease Research Laboratory (VDRL) due to the risk of acquisition of syphilis through blood transfusions. Weight-for-age scores were calculated using the 1990 British Growth References.[16] Moderate wasting was

defined as a weight-for-age z-score <-2 and severe wasting was defined as a weight-for-age z-score <-3. Anaemia was defined according to WHO guidelines.[17] Thrombocytopaenia was defined as a platelet count below 50,000/ml. As CD4 percentages were not available for children aged below five years, absolute CD4 counts were used to define immunodeficiency. Severe immunodeficiency was defined as a CD4 count <1500 cells/µl in children aged < 12months, <750 cells/µl in children aged 1-2 years, <350 cells/µl in children aged 3-4 years and <200 cells/µl in children aged 5 years and older.[18] The prevalence of demographic characteristics and transmission risk factors was calculated. Chi square tests were conducted to assess the association of transmission risk factors with age.

All data were anonymised before being exported to Stata version 15 (StatCorp, Texas, USA) for analysis. The data reported here are part of the national surveillance system and permission was obtained from the Sindh AIDS Control Programme to report the data. Requirement for ethical review was waived by the Ethics Review Committee at the Aga Khan University.

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This study did not receive any funding. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between April 25th and July 15th 2019, 31,239 individuals underwent HIV screening at the Tehsil Hospital Ratodero. The numbers presenting for HIV screening each day peaked to nearly 2000 as the community mobilisation campaign was implemented, dropping to about 100 per day after two months (Figure 1). Over this period, 930 (3.0%) individuals tested HIV positive, of whom 763 (82.0%) were children aged up to 15 years, and 79.2% (604/763) of children were aged 5 years and below. Among the 167 adults aged ≥ 16 years who tested HIV positive, 79.6% (133/167) were female, but among children aged 0-15 years, 60.8% (464/763) of HIV diagnoses were among males. While the numbers who presented for testing dropped over this period, the HIV prevalence remained stable (Figure 1).

Age-group and gender are known for 22,269 of the 31,239 (71%) individuals screened. We estimated HIV prevalence by age group and sex among all 31,239, assuming the distribution of age group and gender was the same in the 8969 whose characteristics were not known as in the 22,269 with data (Table 1). HIV prevalence was highest in the youngest age groups, 0-2 years (7.4%, 283/3803) and 3-5 years (5.9%, 321/5412). In these age groups boys had a higher prevalence than girls (Table 1).

Clinical characteristics of children registered for HIV care

By July 2nd 2019, 591 children had registered for HIV care at the paediatric clinic at the district hospital in Larkana established in response to the outbreak. The median age was 3 years (interquartile range (IQR): 2 - 5 years) and 64% were male (Table 2). More than 90% (394/413) of children who were from the Larkana district lived in Ratodero Tehsil. The median weight-for-age z-score was -3·2 (IQR: -4·7 to -1·9) and 77.2% (393/509) had moderate to severe anaemia, reflecting a high background prevalence of malnutrition and anaemia.[19] The proportion of children with severe immunodeficiency was 14·5% (50/349), and 80.3% (470/585) had Stage 3 or 4 HIV disease using WHO Staging criteria.[20]

The most common reason for classifying children as Stage 3 or 4 were persistent fever and unexplained weight loss and thrombocytopaenia. If severe anaemia and malnutrition are excluded as criteria for WHO staging (given the high background prevalence of these conditions[19]), 24.2% (134/553) had WHO Stage 3 & 4 disease. There was a strong association between CD4 count and WHO stage (with the definition excluding anaemia and malnutrition), with 10.6% (30/283) of children with WHO Stage 1 & 2 disease vs 32.3% (20/62) of children with Stage 3 & 4 disease having severe immunodeficiency as defined by CD4 count ($p<0.001$). Nine of the 591 children who registered for HIV care have subsequently died.

The prevalence of Hepatitis B surface antigen and Hepatitis C antibody positivity was 8·4% (48/574) and 2·6% (15/574) respectively. More boys than girls had Hepatitis B (10·4% (38/366) vs 4·8% (10/208), $p=0·02$ using chi square test), but the opposite was true for

Hepatitis C (1·6% (6/366) vs 4·3% (9/208), p=0·05). Of the five children with thalassemia, two were also VDRL and Hepatitis C antibody positive, most likely acquired through blood transfusion. Hepatitis B surface antibody was not done.

The majority of children (534/584, 91·4%) were taking cotrimoxazole prophylaxis and 66·8% (395/591) were commenced on antiretroviral therapy (ART). The most frequent ART regimen was zidovudine, lamivudine and nevirapine given as a fixed drug combination.

Likely mode of HIV transmission

Mothers of 63% of the children (n=371) and fathers of 52% of the children (n=313) in this clinical cohort were tested for HIV. Of the 371 mothers tested, 39 (10·5%) were HIV positive. Among children aged under one year, seven of 25 (28·0%) mothers were HIV-positive compared to 32 of 346 (9·3%) mothers of children aged one year and older. Eight (2·6%) of the 313 of fathers tested were HIV-positive. Notably, for the 32 HIV-positive mothers for whom an HIV test result was also available for the father, only 6 fathers were HIV-positive. Sixty-four children had at least one HIV-positive sibling. Out of these 64, the mothers of 44 (68·8%) had an HIV test of whom eight (18%) were HIV-positive.

Data on history of multiple injections and blood transfusion were available for 453 children. Nearly 90% gave a history of multiple injections and 40 (8·8%) gave a history of blood transfusions (four of whom had thalassemia, the remainder having received transfusions for iron deficiency anaemia). Multiple injections referred to treatment provided by healthcare practitioners and is distinct from vaccinations distributed through the well-regulated EPI programme. Of the 49 children who reported no history of multiple injections, 34 (69·4%) had a history of blood transfusion. Of the remaining 15 who reported neither blood transfusion nor multiple injections, 12 of the mothers had an HIV test and all were HIV-positive. Table 3 demonstrates the distribution of the 591 children across age, maternal HIV status, multiple injections and transfusion history including those for whom data is missing.

Discussion

This is a preliminary report on an extensive outbreak of HIV infection in Ratodero, Larkana district in Sindh, Pakistan. This outbreak is the latest in a series of outbreaks in Pakistan over the past two decades (Figure 2). However, this is the first time that predominantly children have been infected. More than 700 children were diagnosed with HIV within a 3-month period. Prior to March 2019, there were only 1423 children ever registered and 1041 registered and actively in HIV care across the *whole* country (National AIDS Control Program data). This represents a 54% increase in the cumulative numbers of paediatric diagnoses over the past 13 years, since records of diagnoses were introduced by the National AIDS Control Program.

The age and sex distribution of this most recent outbreak in Ratodero raises important questions regarding the epidemiology of HIV and mode of transmission. It is heavily skewed towards young children under the age of five years, with a predominance of boys (64·5%) in those aged under five years, the reasons for which are unclear. While increased healthcare utilisation by boys may be a possible reason, healthcare seeking behaviour has not shown to be different between genders in rural areas of the province.[21]

One reason put forward to explain this outbreak is the reuse of needles by health practitioners and transfusion of contaminated blood products. Nosocomial outbreaks of HIV infection have been reported in other countries over the past two decades, mostly related to a specific health facility and not previously at this scale.[22-26] Immediately preceding the recognition of the outbreak, 46 children with thalassemia were diagnosed with HIV infection in Larkana at a newly-opened thalassemia service and nearly half were co-infected with HBV. There is low awareness of the need for universal precautions in clinical practice and use of syringes and needles is widespread in both the public and private sector and is the likely reason for the high prevalence of blood borne viruses in the country with the second highest numbers of HCV infections in the world.[27, 28] Pakistan has one of the highest rates of unsafe injections globally which have remained unchanged over the past decade.[29]

In this study, multiple injections that were reported by children in this study were mostly given for treatment of diarrhoeal illnesses and respiratory tract infections. There is a strong preference for parenteral over oral treatment, the latter being construed as less effective among communities.[30] Furthermore, 70% of the population is served by the private health sector, which includes unregulated hospitals, medical general practitioners and a range of informal providers with very few mechanisms to regulate the quality, standards or prices within the private health sector.[31, 32] Blood safety is also problematic with many blood banks unregistered and unregulated.[33] Screening for blood borne viruses by blood banks is erratic and many blood banks regularly utilise paid blood donors with a minority recruiting voluntary blood donors, the former having been shown to have a significantly higher prevalence of HIV infection.[34]

Though cultural practices such as circumcision (males), ear/nose piercing (female children) and head shaving (male and female infants) by barbers using contaminated razors may play a part in transmission of blood borne viruses, preliminary (unpublished) data does not show that these have played a major role in this outbreak.[35] Specifically, the prevalence of these practices was low in the preliminary investigations carried out by the taskforce that conducted preliminary investigations.

Among the 62.7% of mothers of the HIV-positive children who registered for care who did undergo HIV testing, the majority were HIV-negative (89·5%). When stratified by the age of the child, the proportion of HIV-positive infants with a HIV-positive mother was higher (28·0%) compared to older children (9·3% had a HIV-positive mother), suggesting that mother to child transmission (MTCT) may also play a role in transmission in the very young in this outbreak. Interestingly, HIV prevalence was less than 0·05% (1/2990), lower than the national average, in antenatal clinic attendees in 2011 in Larkana,[36] but this estimate may not be representative of the population in that those that attend antenatal care in this setting are likely to be a selected group with higher educational attainment or socioeconomic status.[37]

The term outbreak, defined as the occurrence of disease cases in excess of normal expectancy for a particular time and place, needs to be interpreted with caution in this context. While there was indeed an unexpected increase in the number of cases, 24% of the children who registered for HIV care had WHO Stage 3 or 4 disease which suggests that acquisition of infection was not in the very recent past implying that transmission may have been ongoing for some time, unless this is confounded by a particular virulent sub-type of HIV-1 circulating in the population. This raises the possibility that this outbreak is a spillover of the well-established concentrated HIV subepidemic in key populations in Larkana into the general population. This would be consistent with HIV transmission trends in other regions, particularly eastern Europe, the Russian Federation and central Asia, where transmission began among PWID and subsequently moved into sex worker populations and onward to the general population.[38] Concentrated epidemics in high risk groups, such as PWIDs and sex workers have been reported as early as 2003 in Larkana.[39-43] Importantly, Figure 1 should not be interpreted as a typical epidemic curve for point source outbreaks which demonstrates a rapid increase in the number of cases followed by a somewhat slower decline, and cases tend to fall within one incubation period. While the numbers of individuals being tested on a weekly basis declined, the HIV prevalence remained constant, suggesting that as testing continues more HIV cases will be identified.

In three rounds of the integrated behavioural and biological surveys which were conducted in key populations (PWID, male, female and transgender sex workers) in several districts in Pakistan between 2007 to 2017, the prevalence of HIV in PWID remained relatively stable over the last 10 years in Larkana but there was an upward trend in the HIV prevalence in other risk groups: from 14 to 18% in transgender sex workers, 0 to 4% in male sex workers and 2·5 to 5·0% in female sex workers.[4, 44, 45]

Larkana is known for having a large number of PWID and sex workers,[41] and has the largest male and transgender sex worker population density in Pakistan at 12·4 per 1000 adult men. Approximately 1 in 10 transgender sex workers report injecting drugs and 12% report having sex with PWID,[2] suggesting that transgender sex workers may be a bridging population between PWID and sex work in this area. The city of Larkana is also a frequent stop for truckers and men returning to the rural areas of the country from the larger cities.

The rise in HIV prevalence in female sex workers is also of concern as it signals onward transmission into another key population that had hitherto had low HIV prevalence.

There is no doubt that there is significant potential for an HIV epidemic expansion beyond the key populations across many parts of Pakistan, considering the high HIV prevalence among PWID, low condom usage, risky injecting practises and large population densities of transgender, male and female sex workers in certain cities in Pakistan.[4, 46] The outbreak in a small village in Kot Momin, Sargodha district in Punjab province found a prevalence of 1·3% (35 of 2717 tested) in June 2018. [47] By March 2019 the prevalence had risen to 13·4% (669 of 5000 tested) with reported prevalence higher in women and children although no figures are specified.[35] Findings such as these are of great concern as they demonstrate that bridging of concentrated local subepidemics into the general population in rural areas are already occurring in Pakistan.

There are several limitations which need to be considered in the interpretation of these findings. HIV prevalence data is obtained from a VCT initiative and therefore relies on individuals presenting for or bringing their children for HIV screening. It is therefore not necessarily representative of the burden of HIV infection in the wider community. Due to the large numbers attending for screening and the prevailing panic, there were operational issues including lack of confidentiality and suboptimal pre-test counselling. Parents whose children may have had injections in the past may have been more likely to bring their children for HIV screening. Initial dissemination of information about the outbreak was through widespread media coverage of fourteen children infected, which could have resulted in children preferentially being brought for HIV testing, leading to significant selection bias. Women are usually the main carers of young children so are in turn more likely to be in attendance and be offered an HIV test compared to men in the community. This may explain the finding that most HIV diagnoses in adults were made in females. The prevalence calculations are based on the assumption that the age and gender distribution of people who presented for screening did not change over time, as data on these variables were not recorded

reliably prior to 20 May. For this reason confidence intervals have not been calculated, as they would give a misleading impression of the true confidence.

Other limitations include lack of data on the age and sex of those who attended for screening in the first month and the paucity of data detailing risk practices in adults diagnosed through the screening camps. Comprehensive screening of mothers of newly HIV-positive children was also not undertaken which would have helped to elucidate the extent to which mother-to-child transmission played a role. The low numbers of mothers tested is likely due to lack of sufficient awareness and training of HIV care providers and therefore mothers not being routinely tested and/or maternal testing having been performed but not having been systematically entered into the clinical records. The use of routine clinical data is limited by missing data and also possible inaccuracies in coding of WHO HIV stage.

The children who registered for care had advanced disease, a high prevalence of Hepatitis B and C co-infection, tuberculosis, malnutrition and anaemia. Hepatitis C viral load data were not available so chronicity of infection and need for treatment could not be ascertained. Only 67% commenced ART because adequate drug supplies and trained staff were not available. ART regimen was determined by age. Tenofovir was not given to children below 3 years or age or those weighing below 20kg and were therefore given zidovudine, lamivudine and nevirapine. Clinicians were aware that this may potentially result in Hepatitis B being treated with monotherapy but no alternatives were available. Extensive training of various cadres of healthcare workers is required to support provision of long term HIV care. In addition, procurement of HIV diagnostics and appropriate drug formulations and establishment of robust supply chains is needed. There is an urgent need to invest in improvement of blood safety services and infection control and regulation of health care providers. Studies to understand the full extent of the outbreak and its drivers will guide specific HIV prevention strategies. In addition, population-level surveys to estimate the burden on blood-borne viruses are also needed. Epidemiological and phylogenetic studies are underway and will help to shed light on the evolution and propagation of this HIV outbreak. Importantly

epidemiological surveillance needs to be strengthened and involvement of communities should be a critical component of the response if the HIV epidemic in Pakistan is to be controlled.

Contributors: FM1 and RAF conceived the study. SM, JS and SS provided the necessary data. FM1, SS, JS, AA and AL cleaned the data. FM1, AAN, AL and VS conducted data analyses. FM1, FM2, RS, SB, FNQ, SBS, AMK, SHA, SAM, SAS and RAF were involved in outbreak investigation. FM1, PK and RAF prepared the report and all authors contributed to the report and approved the final draft for submission. FM had full access to all the data in the study and had final responsibility for the decision to submit for publication.

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References

1. Pakistan country fact sheet 2016. United Nations Program on HIV/AIDS. Geneva, Switzerland: UNAIDS 2018.
2. Reza T, Melesse DY, Shafer LA, Salim M, Altaf A, Sonia A, et al. Patterns and trends in Pakistan's heterogeneous HIV epidemic. Sexually transmitted infections. 2013; **89 Suppl 2**(Suppl 2): ii4-ii10.
3. Bergenstrom A, Achakzai B, Furqan S, ul Haq M, Khan R, Saba M. Drug-related HIV epidemic in Pakistan: a review of current situation and response and the way forward beyond 2015. Harm Reduct J. 2015; **12**: 43-.
4. Second Generation HIV Surveillance Round 5: Integrated Biological and Behavioural Surveillance in Pakistan 2016-2017 Islamabad, Pakistan: National AIDS Control Program; 2017.
5. Melesse DY, Shafer LA, Emmanuel F, Reza T, Achakzai BK, Furqan S, et al. Heterogeneity in geographical trends of HIV epidemics among key populations in Pakistan: a mathematical modeling study of survey data. J Glob Health. 2018; **8**(1): 010412-.

6. Melesse DY, Blanchard J. The Future of HIV in Pakistan: modeled Projections of Pakistan's Epidemic. Islamabad, Pakistan: National AIDS Control Program and Canada-Pakistan HIV AIDS Surveillance Project; 2011.
7. Hawkes S, Collumbien M, Platt L, Lalji N, Rizvi N, Andreasen A, et al. HIV and other sexually transmitted infections among men, transgenders and women selling sex in two cities in Pakistan: a cross-sectional prevalence survey. Sex Transm Infect. 2009; **85 Suppl 2**: ii8-16.
8. Mayhew S, Collumbien M, Qureshi A, Platt L, Rafiq N, Faisel A, et al. Protecting the unprotected: mixed-method research on drug use, sex work and rights in Pakistan's fight against HIV/AIDS. Sex Transm Infect. 2009; **85 Suppl 2**: ii31-6.
9. Mishra S, Thompson LH, Sonia A, Khalid N, Emmanuel F, Blanchard JF. Sexual behaviour, structural vulnerabilities and HIV prevalence among female sex workers in Pakistan. Sexually transmitted infections. 2013; **89 Suppl 2**(Suppl 2): ii34-ii42.
10. Khanani MR, Somani M, Rehmani SS, Veras NM, Salemi M, Ali SH. The spread of HIV in Pakistan: bridging of the epidemic between populations. PLoS One. 2011; **6**(7): e22449.
11. Ahmad S, Mehmood J, Awan AB, Zafar ST, Khoshnood K, Khan AA. Female spouses of injection drug users in Pakistan: a bridge population of the HIV epidemic? East Mediterr Health J. 2011; **17**(4): 271-6.
12. Province wise provisional results of Census 2017 Pakistan: Pakistan Bureau of Statistics; 2017.
13. Socio-economic survey of Ratodero Taluka, District Larkana, Sindh. Islamabad, Pakistan: Rural Support Programmes Network; 2008.
14. Arif F. HIV crisis in Sindh, Pakistan: the tip of the iceberg. Lancet Infect Dis. 2019; **19**(7): 695-6.
15. Pakistan Country Strategy for HIV Testing and Counselling based on situation and response analysis Islamabad, Pakistan: National AIDS Control Program; 2013.
16. Cole TJ. Growth monitoring with the British 1990 growth reference. Arch Dis Child. 1997; **76**(1): 47-9.
17. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. Vitamin and Mineral Nutrition Information System (WHO/NMH/NHD/MNM/11.1). Geneva, Switzerland: World Health Organization; 2011.
18. Laboratory guidelines for enumerating CD4 T lymphocytes in the context of HIV/AIDS. New Delhi, India: World Health Organization Regional Office for South-East Asia; 2007.
19. Habib MA, Black K, Soofi SB, Hussain I, Bhatti Z, Bhutta ZA, et al. Prevalence and Predictors of Iron Deficiency Anemia in Children under Five Years of Age in Pakistan, A Secondary Analysis of National Nutrition Survey Data 2011-2012. PLoS One. 2016; **11**(5): e0155051.
20. WHO case definitions of HIV for surveillance and revised clinical staging and immunological classification of HIV-related disease in adults and children. Geneva, Switzerland: World Health Organization; 2006.
21. Nuruddin R, Hadden WC, Petersen MR, Lim MK. Does child gender determine household decision for health care in rural Thatta, Pakistan? Journal of Public Health. 2009; **31**(3): 389-97.
22. Vun MC, Galang RR, Fujita M, Killam W, Gokhale R, Pitman J, et al. Cluster of HIV Infections Attributed to Unsafe Injection Practices--Cambodia, December 1, 2014-February 28, 2015. MMWR Morbidity and mortality weekly report. 2016; **65**(6): 142-5.
23. Yerly S, Quadri R, Negro F, Barbe KP, Cheseaux JJ, Burgisser P, et al. Nosocomial outbreak of multiple bloodborne viral infections. J Infect Dis. 2001; **184**(3): 369-72.
24. Morris K. Transfusion-related HIV outbreak in Kazakhstan children. Lancet Infect Dis. 2006; **6**(11): 689.

25. El Sayed NM, Gomatos PJ, Beck-Sague CM, Dietrich U, von Briesen H, Osmanov S, et al. Epidemic transmission of human immunodeficiency virus in renal dialysis centers in Egypt. J Infect Dis. 2000; **181**(1): 91-7.
26. Pan X, Jiang J, Ma Q, Zhang J, Yang J, Chen W, et al. Outbreak of HIV Infection Linked to Nosocomial Transmission, China, 2016-2017. Emerging infectious diseases. 2018; **24**(12): 2141-9.
27. Janjua NZ, Razaq M, Chandir S, Rozi S, Mahmood B. Poor knowledge--predictor of nonadherence to universal precautions for blood borne pathogens at first level care facilities in Pakistan. BMC infectious diseases. 2007; **7**: 81-.
28. Ahsan A, Khan AZ, Javed H, Mirza S, Chaudhary SU, Shahzad-Ul-Hussan S. Estimation of hepatitis C prevalence in the Punjab province of Pakistan: A retrospective study on general population. PloS one. 2019; **14**(4): e0214435-e.
29. Hayashi T, Hutin YJF, Bulterys M, Altaf A, Allegranzi B. Injection practices in 2011-2015: a review using data from the demographic and health surveys (DHS). BMC health services research. 2019; **19**(1): 600-.
30. Ali SA, Donahue RMJ, Qureshi H, Vermund SH. Hepatitis B and hepatitis C in Pakistan: prevalence and risk factors. International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases. 2009; **13**(1): 9-19.
31. Akbari AH, Rankaduwa W, Kiana AK. Demand for Public Health Care in Pakistan. The Pakistan Development Review. 2009; **48**: 141-53.
32. Shaikh BT. Private sector in health care delivery: A reality and challenge in Pakistan. Journal of Ayub Medical College, Abbottabad : JAMC. 2015; **27**(2): 496-8.
33. Sultan S, Irfan SM, Siddiqui M, Zaidi SM. Current trends of seroprevalence of transfusion transmitted infections in Pakistani beta-thalassaemic patients. The Malaysian journal of pathology. 2016; **38**(3): 251-5.
34. Alaei K, Sarwar M, Alaei A. The Urgency to Mitigate the Spread of Hepatitis C in Pakistan Through Blood Transfusion Reform. Int J Health Policy Manag. 2018; **7**(3): 207-9.
35. Wahid B. An update on the severe outbreak of HIV in Kot Imrana, Pakistan. Lancet Infect Dis. 2019; **19**(3): 241.
36. Antenatal serosurveillance for HIV/AIDS in Pakistan 2011. Islamabad, Pakistan: National AIDS Control Program; 2011.
37. Sahito A, Fatmi Z. Inequities in Antenatal Care, and Individual and Environmental Determinants of Utilization at National and Sub-national Level in Pakistan: A Multilevel Analysis. Int J Health Policy Manag. 2018; **7**(8): 699-710.
38. Niccolai LM, Shcherbakova IS, Toussova OV, Kozlov AP, Heimer R. The potential for bridging of HIV transmission in the Russian Federation: sex risk behaviors and HIV prevalence among drug users (DUs) and their non-DU sex partners. J Urban Health. 2009; **86 Suppl 1**: 131-43.
39. Altaf A, Pasha S, Vermund SH, Shah SA. A second major HIV outbreak in Larkana, Pakistan. JPMA The Journal of the Pakistan Medical Association. 2016; **66**(12): 1510-1.
40. Memon A, Haider S, Altaf A. Alarming increase in reported HIV cases from Larkana, Pakistan: a matter of serious concern. JPMA The Journal of the Pakistan Medical Association. 2014; **64**(2): 205-6.
41. Altaf A, Zahidie A, Agha A. Comparing risk factors of HIV among hijra sex workers in Larkana and other cities of Pakistan: an analytical cross sectional study. BMC Public Health. 2012; **12**: 279.
42. ur Rehman N, Emmanuel F, Akhtar S. HIV transmission among drug users in Larkana, Pakistan. Trop Doct. 2007; **37**(1): 58-9.

43. Shah SA, Altaf A, Mujeeb SA, Memon A. An outbreak of HIV infection among injection drug users in a small town in Pakistan: potential for national implications. International journal of STD & AIDS. 2004; **15**(3): 209.
44. HIV Second generation surveillance in Pakistan: National report Round II 2006-2007. Islamabad, Pakistan: National AIDS Control Program; 2007.
45. HIV Second Generation Surveillance in Pakistan: National Report Round IV, 2011. Islamabad, Pakistan: National AIDS Control Program; 2011.
46. Ansari JA, Salman M, Safdar RM, Ikram N, Mahmood T, Zaheer HA, et al. HIV/AIDS outbreak investigation in Jalalpur Jattan (JPJ), Gujrat, Pakistan. Journal of epidemiology and global health. 2013; **3**(4): 261-8.
47. Zaid M, Afzal MS. HIV outbreak in Pakistan. The Lancet Infectious diseases. 2018; **18**(6): 601.

Table 1: Estimated HIV prevalence by age and sex

Age (Years)	Male			Female			Total		
	Known screened	Estimated total screened	HIV-positive (estimated prevalence)	Known screened	Estimated total screened	HIV-positive (estimated prevalence)	Known screened	Estimated total screened	HIV-positive (estimated prevalence)
≤ 2	1446	2029	168 (8·3%)	1264	1774	115 (6·5%)	2710	3803	283 (7·4%)
3 to 5	2034	2853	209 (7·3%)	1824	2559	112 (4·4%)	3858	5412	321 (5·9%)
6 to 15	3073	4311	87 (2·0%)	3237	4541	72 (1·6%)	6310	8851	159 (1·8%)
16 to 49	2190	3072	30 (1·0%)	5831	8179	118 (1·4%)	8021	11251	148 (1·3%)
≥ 50	268	376	4 (1·0%)	1102	1546	15 (1·0%)	1370	1922	19 (1·0%)
Total	**9011**	**12641**	**498 (3·9%)**	**13258**	**18598**	**432 (2·3%)**	**22269**	**31239**	**930 (3·0%)**

Table 2: Sociodemographic and clinical characteristics of 591 HIV-positive children registered for care in Larkana between April 24-July 2, 2019

Characteristic	N (%)	Missing data
Socio-demographic		
Age (years) <1 1 2 3-5 6-10 >10	 32 (5·4%) 73 (12·4%) 112 (19·0%) 261 (44·2%) 99 (16·8%) 14 (2·3%)	0
Male	379 (64·1%)	0
District Larkana Jacobabad Qamber Shahdadkot Shikarpur Other[a]	 423 (73·6%) 24 (4·2%) 36 (6·2%) 88 (15·3%) 4 (0·7%)	16
In education (in children aged >5 years) (n=108) Primary Secondary No schooling	 18 (16·7%) 5 (4·6%) 85 (79·0%)	5
Risk factors		
Mother HIV antibody positive	39 (10·5%)	220
Father HIV antibody positive	8 (2·6%)	278
Sibling HIV status ≥1 sibling HIV-positive All siblings HIV-negative	 64 (19·9%) 257 (80·1%)	270[b]
History of blood transfusion	40 (8·8%)	138
History of multiple injections	404 (89·2%)	138
Clinical		
Thalassemia[c]	5 (0·9%)	
Hepatitis B surface antigen positive	48 (8·4%)	17
Hepatitis C antibody positive	15 (2·6%)	17
Anaemia Not anaemic Mild Moderate Severe	 49 (9·6%) 67 (13·2%) 331 (65·0%) 62 (12·2%)	82
Thrombocytopaenia	29 (5·8%)	91
Presumed tuberculosis[d]	38 (6·5%)	8
Weight for age z-score >-2 -2 to -3 (wasting)	 154 (26·1%) 121 (20·5%)	1

Characteristic	N (%)	Missing data
<-3 (severe wasting)	315 (53·4%)	
HIV WHO stage 3 or 4[e]	134 (24·2%)	38
Severe immunodeficiency	50 (14·5%)	246
Receiving cotrimoxazole prophylaxis	534 (91·4%)	7
Receiving isoniazid prophylaxis	2 (0·4%)	18
Initiated on antiretroviral therapy	395 (66·8%)	
ART Regimen (n=395)		
Zidovudine, Lamivudine, Nevirapine	317 (80·3%)	
Tenofovir, Lamivudine, Efavirenz	60 (15·2%)	
Tenofovir, Lamivudine, Nevirapine	10 (2·5%)	
Abacavir, Lamivudine, Nevirapine	8 (2.0%)	

[a] Naushahro Feroze=1; Ghotki=2; Dadu=1

[b]128:no siblings tested; 50: patient had no siblings; 92: missing data

[c]2 not tested for VDRL

[d]Pakistan Pediatric Association (PPA) score (a modified form of Keith Edwards score) of 7

[e]If wasting and anaemia not included as criteria for WHO Staging

Table 3: Distribution by age of possible risk factors for HIV infection in children who registered for HIV care

Age group	History of multiple injections	Yes		No		Unknown	
	History of transfusion	Yes	No	Yes	No	Unknown	**Total**
	Mother's HIV status						
0-11 months	Positive	0	3	1	3	0	**7**
	Negative	0	14	0	0	4	**18**
	Unknown	0	3	1	0	3	**7**
1-2 years	Positive	0	7	2	5	0	**14**
	Negative	0	71	4	0	29	**104**
	Unknown	1	50	2	1	13	**67**
3-4 years	Positive	0	6	0	1	0	**7**
	Negative	1	83	7	0	27	**118**
	Unknown	1	55	5	1	21	**83**
5-9 years	Positive	0	7	0	2	0	**9**
	Negative	3	50	7	0	15	**75**
	Unknown	0	29	4	1	16	**50**
10-14 years	Positive	0	1	0	1	0	**2**
	Negative	0	13	0	0	4	**17**
	Unknown	0	6	1	0	6	**13**
	Total	**6**	**398**	**34**	**15**	**138**	**591**

Research in context

Evidence before this study

In April 2019, a large number of cases of HIV infection mainly in children were reported in Sindh, Pakistan On July 29, 2019, we searched PubMed for articles published between Jan 1, 2000 and July 15, 2019 with the terms “HIV” and “Pakistan” in the title. We found 250 publications, including 7 news items, 61 letters and perspective articles and the remainder research studies related to HIV infection. The studies highlight a high prevalence of HIV infection among certain groups including persons who inject drugs, male, female and transgender sex workers in Pakistan, with significant geographical heterogeneity across the country. Several nosocomial outbreaks of HIV infection have been reported in adults in the context of poor infection control practices and unscreened blood in healthcare settings. Children have not been reported as a significant population affected and we identified no detailed report of the 2019 paediatric HIV outbreak in Sindh.

Added value of this study

In this study, we present a detailed report of an extensive outbreak of HIV infection in Sindh, Pakistan in 2019. This epidemic is characterised by an unusually high proportion of paediatric cases. Likely sources of infection include contaminated needles and blood products and mother-to-child transmission. Many of the children appear to have advanced disease which raises the question regarding the recency of infection and the possibility of spillover of the well-established concentrated HIV subepidemic in key populations in Larkana into the general population.

Implications of all the available evidence

This is the most extensive outbreak of paediatric HIV infection ever reported in Pakistan with the true extent unknown. Training of healthcare workers on paediatric HIV care and provision of drugs for treatment of HIV and blood-borne viruses is an urgent priority. There is a need to improve infection control practices and blood safety. Investigation of the drivers of the epidemic will form the basis for improved HIV management and control in both the province and nationally.