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## Inside



# Impact of Hepatitis B vaccination programme in Bangladesh

Hepatitis B virus infection is a leading cause of morbidity and mortality due to hepatocellular carcinoma and liver cirrhosis worldwide. During 2003-2005 Bangladesh introduced hepatitis B vaccine into the routine childhood vaccination schedule. This study evaluated the impact of hepatitis B vaccine introduction in Bangladesh by comparing hepatitis B surface antigen (HBsAg) prevalence among children born before and children born after hepatitis B vaccine introduction. We selected a nationally representative sample of 2,100 children from both pre-vaccine era and vaccine era children from 105 randomly selected *mouzas* (geographic units) of Bangladesh. We collected a blood sample from each child along with vaccination and demographic information. All samples were tested for antibody to hepatitis B core antigen (anti-HBc); anti-HBc-positive samples were further tested for HBsAg. One hundred eight (5.1%) pre-vaccine and 16 (0.8%) vaccine era children were anti-HBc-positive. Of the pre-vaccine era children, 26 (1.2%) children and of the vaccine era children 1 (<0.1%)

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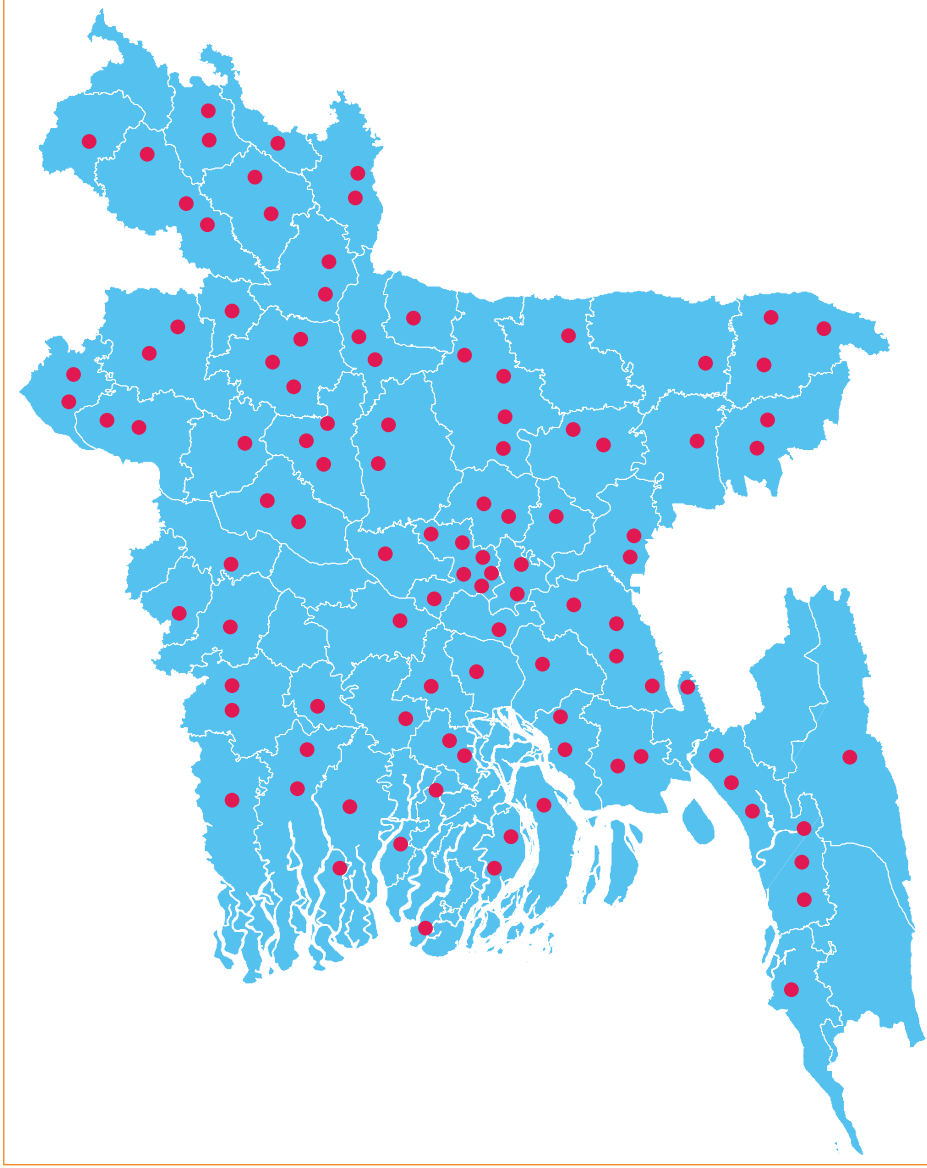
**child were HBsAg-positive. Study results suggest that the hepatitis B vaccination programme in Bangladesh is highly effective.**

Globally, hepatitis B virus (HBV) infection is a leading cause of morbidity and mortality due to hepatocellular carcinoma and liver cirrhosis (1). While data on the hepatitis B disease burden in Bangladesh is limited, some previous small-scale studies suggest that hepatitis B surface antigen (HBsAg) prevalence ranges from 3-7% among the general population (2-5) and 1.5-12% among children under 5 years (2,3,5). Bangladesh introduced hepatitis B vaccine in a phased manner during 2003-2005 into the routine childhood vaccination schedule provided at 6, 10, and 14 weeks of age. The objective of introducing the vaccine in Bangladesh was to reduce hepatitis B disease burden by 80%. However, the widely recommended hepatitis B vaccine birth dose is not administered in the childhood vaccination schedule in Bangladesh, where 71% of births occur at home (6). This study was designed to evaluate the impact of the introduction of hepatitis B vaccine in Bangladesh by comparing HBsAg prevalence among children born before and after hepatitis B vaccine introduction.

We randomly selected a nationally representative sample of 2,100 children in two age groups, one group that was born before the introduction of hepatitis B vaccine, and another group that was born immediately after the introduction of the vaccine. According to the 2001 census, there are 63,650 *mouzas* (geographic units) in Bangladesh, each with an average population of about 2,000 people. We randomly selected 105 *mouzas* as the survey clusters from the list of *mouzas* using the probability proportional to size cluster sampling approach. Selected survey clusters were distributed all over Bangladesh (Figure 1). From each selected cluster, we selected 20 children from the pre-vaccine era group who were born between 1 April 2001 and 31 March 2002, and 20 children from the vaccine era group who were born between 1 November 2005 and 31 October 2006.

A cross-sectional house-to-house survey was conducted during September 2011 to April 2012. In each cluster, two field teams, each comprised of one interviewer and one phlebotomist, worked simultaneously to search for eligible children. One team searched from the centre of the cluster in a randomly chosen direction to the boundary of the cluster, while the other team searched from the opposite direction. They visited all the households until they found 20 pre-vaccine era and 20 vaccine era children. Only one child was selected from a household. In each cluster, two sample collection centres were usually set up temporarily in Expanded Programme on Immunization (EPI)/community clinics or in houses of local leaders. Guardians of selected children were requested to bring their children to these sample collection centres.

*Figure 1: Map of Bangladesh showing clusters selected for the study*



Phlebotomists collected a 5 ml venous blood sample from children after obtaining written consent from guardians. All safety measures were followed during sample collection. All children who provided blood samples were provided with their blood grouping as a benefit from this study. Interviewers collected information on children's demographics, vaccination history, and

possible risk exposures for HBV infection. Vaccination history was obtained from children's vaccination cards or by their guardians' reports if vaccination cards were unavailable. At the end of each day, phlebotomists processed the samples at the local health complex and stored them at 2-4°C in the refrigerator of the Upazila Health Complex. All samples were transported to the laboratory of the Institute of Epidemiology, Disease Control and Research (IEDCR) in Dhaka within one week of collection.

Samples were tested at the IEDCR laboratory. Since, in general, HBsAg carriers are also positive for antibody to hepatitis B core antigen (anti-HBc), all samples were first tested for anti-HBc and all anti-HBc-positive samples were then tested for HBsAg (7,8). ELISA kits manufactured by DiaSorin, Italy, were used to test for anti-HBc and ELISA kits manufactured by bioMérieux, France, were used to test for HBsAg. As part of quality assurance of laboratory testing, all HBsAg-positive samples and a subset of remaining samples were also tested for HBsAg in the Division of Viral Hepatitis Laboratory of the Centers for Disease Control and Prevention, Atlanta, USA.

We computed descriptive statistics of socio-demographic variables and other characteristics of the sampled population. We used cluster adjusted standard errors to account for cluster effect in estimating 95% confidence intervals for HBV infection rates and odds ratios for potential risk factors of infection. Bivariate analysis was performed to identify the potential risk factors for being HBsAg-positive among pre-vaccine era children. Chi-square tests were used to examine the relationship between independent variables and the outcome variable in the bivariate analysis.

The field team approached 2,203 pre-vaccine era children and 2,270 vaccine era children to enroll 2,100 children from each group. Five percent of pre-vaccine era children and 8% of vaccine era children refused to provide blood samples. In both groups, approximately 51% of children were male. Forty-four percent of the mothers of pre-vaccine era children and 56% of the mothers of vaccine era children had completed primary education (Table 1).

The EPI vaccination card retention rate was higher among vaccine era children. Forty-five percent of guardians of vaccine era children and 17% of guardians of pre-vaccine era children showed the vaccination cards for their children during household visits by the field team members. According to vaccination card information or report by guardians, 94% of vaccine era children received all three doses of hepatitis B vaccine. However, 1.6% of guardians of vaccine era children could not recall the vaccination status of their children. On the other hand, 3.3% of guardians of pre-vaccine era children mentioned that hepatitis B vaccine, obtained from commercial sources, was provided to their children and 5.1% of guardians of pre-vaccine era children could not recall the hepatitis B vaccination status of their children (Table 2).

*Table 1: Demographic characteristics of children participating in the study*

| Characteristics | Pre-vaccine era children[1] N=2,100 | Vaccine era children[2] N=2,100 |
|---|---|---|
| Male (%) | 1,086 (51.7) | 1,077 (51.3) |
| Mother completed at least primary education (%) | 928 (44.2) | 1,174 (55.9) |
| Mean number of household members (interquartile range) | 5.5 (4-6) | 5.4 (4-6) |
| Monthly mean household expenditure in taka in thousands (interquartile range) | 8.1 (5.1-10.0) | 7.5 (4.5-9.0) |

[1]Born between 1 April 2001 and 31 March 2002
[2]Born between 1 November 2005 and 31 October 2006

*Table 2: Hepatitis B vaccination status of children participating in the study*

| Hepatitis B vaccination | Pre-vaccine era children N=2,100 n (%) | Vaccine era children N=2,100 n (%) |
|---|---|---|
| Vaccination card available | 353 (16.8) | 953 (45.4) |
| Received all 3 doses of vaccine* | 69 (3.3) | 1,976 (94.1) |
| 1st dose* | 80 (3.8) | 2,031 (96.7) |
| 2nd dose* | 76 (3.6) | 2,012 (95.8) |
| 3rd dose* | 69 (3.3) | 1,978 (94.2) |
| Unable to recall the doses of hepatitis B vaccine | 107 (5.1) | 34 (1.6) |

*According to vaccination card or guardian's reporting

According to laboratory results, 108 (5.1%) pre-vaccine era children and 16 (0.8%) vaccine era children were positive on anti-HBc ELISA testing. Twenty-six (1.2%) children of pre-vaccine era group and 1 (<0.1%) child of vaccine era group were positive on HBsAg ELISA testing (Table 3). In the households of HBsAg-positive children, no other family members were known to have HBV infection. In the risk factor analysis for HBV infection of pre-vaccine era children, no potential exposures were significantly associated with HBsAg positivity (Table 4).

*Table 3: Hepatitis B seroprevalence among children participating in the study*

| Test | Pre-vaccine era children N=2,100 n (%) | Vaccine era children N=2,100 n (%) |
|---|---|---|
| Anti-HBc | 108 (5.1) (95% CI: 3.8-6.5) | 16 (0.08) (95% CI: 0.03-1.2) |
| HBsAg | 26 (1.2) (95% CI: 0.7-1.7) | 1 (<0.1) (95% CI: 0-0.3) |

*Table 4: Bivariate analysis for selected risk factors for being HBsAg-positive among pre-vaccine era children*

| Characteristics | HBsAg -positive children N=26 n (%) | HBsAg -negative children N=2,074 n (%) | Odds ratio (95% CI) | p-value |
|---|---|---|---|---|
| **History of surgery** | | | | |
| No | 21 (81) | 1,858 (90) | 1 | |
| Yes | 5 (19) | 216 (10) | 2.0 (0.8-5.3) | 0.15 |
| **Previously taken to a dentist** | | | | |
| No | 25 (96) | 1,947 (97) | | |
| Yes | 1 (4) | 127 (6) | 0.6 (0.1-4.5) | 0.63 |
| **Circumcised (males only)[1]** | | | | |
| No | 4 (33) | 333 (31) | 1 | |
| Yes | 8 (67) | 740 (69) | 0.9 (0.3-3.1) | 0.87 |
| **Previously received an injection (except EPI vaccination)** | | | | |
| No | 12 (46) | 1,119 (54) | 1 | |
| Yes | 14 (54) | 955 (46) | 1.4 (0.6-3.2) | 0.47 |
| **Shared needles for ear, nose, and/or body piercing (females only)[2]** | | | | |
| No | 10 (71) | 1,817 (90) | 1 | |
| Yes | 4 (29) | 206 (10) | 2.1 (0.6-7.8) | 0.25 |

[1]N= 12 for HBsAg-positive cases; N=1,073 for HBsAg-negative cases
[2]N= 14 for HBsAg-positive cases; N=1,001 for HBsAg-negative cases

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***Comments***

Hepatitis B vaccine coverage is high among children in the cohort born immediately after the introduction of hepatitis B vaccine in Bangladesh. The study results suggest that even without a birth dose, the hepatitis B vaccine programme in Bangladesh is highly effective. Efforts should be continued to maintain the high child vaccination coverage against HBV infection. These findings support the continued investment in the hepatitis B vaccination programme in Bangladesh.

We report two potential study limitations. First, vaccination cards were not available for a large number of children. In those cases, vaccination status of children was reported by guardians and might have been subject to recall error. Second, in the bivariate analysis, no potential exposures were significantly associated with HBsAg positivity among pre-vaccine era children. This finding might be because of the limited number of HBsAg-positive samples to report the potential risk factors for HBV infection. However, further studies are required to better define the transmission dynamics and risk factors of HBV infection among children in Bangladesh.

This study provides a useful baseline for future assessment of the impact of childhood hepatitis B immunization in Bangladesh. In the absence of routine surveillance to monitor the impact of the hepatitis B childhood vaccination programme in Bangladesh, we recommend that a representative HBsAg serosurvey be conducted regularly to evaluate the impact of the programme. A simple and inexpensive rapid test for HBsAg has been found to provide results that are comparable to those obtained using standard ELISA testing in laboratories (9,10). Moreover, a recent national level hepatitis B serosurvey conducted in Cambodia suggested that rapid tests were feasible in a developing country setting with minimal laboratory infrastructure and limited financial resources (11). The rapid HBsAg test might be useful in future serosurveys given the expense and the logistical difficulties of collecting intravenous blood samples and maintaining the cold chain of samples for laboratory ELISA testing. Furthermore, since Bangladesh does not have any nationally representative data on HBV infection among the adult population, we also recommend consideration of a national hepatitis B serosurvey among adults using rapid HBsAg tests.

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# Continued household faecal contamination following a sanitation intervention in rural Bangladesh

**We provided potties and a customized hoe-like tool (sani-scoop) to dispose of child and animal faeces to 104 rural households with children under three years of age. Despite the reported use of the potties (67%) and sani-scoops (89%), minimal differences were observed at a three-month follow-up visit with regards to the presence of human faeces near households (20%; 19/96) compared to baseline (16%; 16/104). Similarly, there were minimal differences in the presence of animal faeces observed in the household compounds at the three-month follow-up visit (84%; 87/104) compared to baseline (96%; 92/96). Barriers to the effective use of the distributed hardware included insufficient potty training, inconsistent use of the hardware and the perception that animal faeces are harmless. Practical strategies need to be developed for safe disposal of faeces around the household to reduce the faecal contamination of a child's household environment.**

Diarrhoeal diseases are one of the most important causes of mortality in children under 5 years of age. Enteropathogens causing diarrhoeal illnesses are most commonly transmitted inside households where children live (1). Pathogens such as rotavirus and *Vibrio cholerae* cause severe diarrhoea in young children and are known to be transmitted through human faeces while pathogens such as *Campylobacter jejuni* and *Giardia spp.,* have been isolated from both animal and human faeces (2-5). Several studies have shown that open defaecation and the unsafe disposal of faeces are associated with an increased risk of diarrhoeal disease (6,7). However, common practices of either leaving faeces in or around the household compound or disposing of faeces in unsafe ways, such as throwing them into nearby bushes (conversation with Sultana R, Assistant Scientist, icddr,b, January 2013), remain difficult behaviours to modify. In rural Bangladesh, child and animal faeces are often found in and around households even where latrines are available (8).

To promote the safe disposal of human and animal faeces from households and compounds, we provided two kinds of sanitation hardware, child potties and a hoe-like tool (sani-scoop), to 104 rural households having at least one child under 3 years of age. We measured the impact of providing the sanitation hardware on the presence of faecal contamination in the household environment, which included the area near the home and the adjoining courtyard.

The study was conducted from January to April 2011 in rural Kishoreganj. Field workers conducted a baseline survey and a three-month follow-up survey which included questions regarding demographics, household size, socio-economic status and an observational assessment for the presence of animal and human faeces in rural compounds where several households share a common courtyard. The presence of human faeces near the household was defined as any faeces observed close to or behind the household structure and in any portion of the adjoining courtyard that the household members reported they were responsible for cleaning. Observations within a compound identified human and animal faeces within the courtyard, around and behind other compound households, but not inside household structures.

Each household enrolled in the study was given a potty and a sani-scoop to facilitate the hygienic disposal of child faeces into latrines and facilitate the disposal of animal faeces into designated pits. Community health promoters (CHPs) visited the study households three times a month during the study to encourage the use of the sanitation hardware. The CHPs conducted household visits and used behaviour change communication materials, including a flip-chart and cue cards showing pictures and text messages, to educate primary caregivers and other members of families on how to introduce potties to children, how to use sani-scoops, and how to keep the hardware clean. The CHPs delivered messages that included the health benefits of keeping the household environment clean as well as other benefits of doing so, such as the social acceptance of cleanliness among neighbours.

In the follow-up survey, participants were asked how often they used the sanitation hardware and field workers made observations to assess the presence and condition of the hardware and assess the presence of human and animal faeces.

The field workers surveyed 104 households at baseline and 96 households at follow-up. Many respondents reported using the potties for child defaecation events (63/75; 84%) and using the sani-scoops for disposal of animal faeces (85/96; 89%). A significantly lower proportion of children's last defaecation events were reported to have occurred in the courtyard at follow-up (4/75, 5%) compared to baseline (47/102, 46%; $p<.001$) and a significantly greater proportion of defaecation events were reported to have occurred in potties at follow-up (63/96, 84%) than at baseline (15/104, 15%; $p<.001$). However, no statistically significant differences were observed in the presence of human faeces near the intervention households at follow-up (6/96, 6%) than at baseline (13/104, 13%; $p=0.17$). Observed animal faeces were significantly more prevalent in intervention compounds at follow-up (92/96, 96%) compared to baseline (87/104. 84%; $p=0.003$) (Table 1).

*Table 1: Changes in reported child sanitation practices and observations of faeces at baseline and at three months among households that received sanitation hardware (potties and sani-scoops), Kishoreganj, Bangladesh, 2011*

| Child sanitation practices | Sanitation practices in intervention communities at time points | | |
|---|---|---|---|
| | Baseline n (%) | 3 month follow-up n (%) | Change Difference % (p-value) |
| **Last reported defaecation site of child under 3 years** | **N=104** | **N=75** | |
| Potty | 15 (15) | 63 (84) | 69 (<0.001) |
| Courtyard | 47 (46) | 4 (5) | -41 (<0.001) |
| Inside house | 22 (22) | 1 (1) | -21 (<0.001) |
| In open space outside front yard | 12 (12) | 2 (3) | -9 (0.03) |
| In bush/jungle | 1 (1) | 0 (0) | -1 (0.2) |
| In toilet | 2 (2) | 3 (4) | 2 (0.4) |
| In katha/cloth | 3 (3) | 2 (3) | 0 (1) |
| **Faeces disposal practice** | | | |
| Not disposed | 2 (2) | 0 (0) | -2 (0.23) |
| Disposed elsewhere | 98 (96) | 72 (96) | 0 (1) |
| Disposed with sani-scoop | N/A | 0 (0) | - |
| **Faeces disposal site** | | | |
| In bush/jungle | 42 (41) | 8 (11) | -30 (<0.001) |
| In latrine | 25 (25) | 55 (73) | 48 (<0.001) |
| In drain | 14 (14) | 7 (9) | -5 (0.44) |
| In garbage | 13 (13) | 1 (1) | -12 (0.003) |
| In the open | 2 (2) | 0 (0) | -2 (0.39) |
| **Observation of open defaecation** | **N=104** | **N=96** | |
| Human faeces within the compound | 16 (15) | 19 (20) | 4 (0.46) |
| Human faeces near the household | 13 (13) | 6 (6) | -6 (0.17) |
| Animal faeces within the compound | 87 (84) | 92 (96) | 12 (0.003) |

To further understand why the intervention had limited effect, we conducted a qualitative investigation from July to September 2011. We created three categories of households based on the amount of observed change (none or minimal, some or average, and high) in the presence of human and animal faeces following the intervention. We purposively selected 40 participants from each of the three categories of households to conduct in-depth interviews and focus group discussions with household members, including primary caregivers. We considered variations in types of households with respect to compound size and number of animals owned, to identify barriers to using the sanitation hardware and to explore underlying reasons for the lack of observed change in the presence of faeces in the household environment.

During the in-depth interviews, participants reported insufficient potty training, inconsistent potty use, and delays in disposing faeces due to other household tasks as barriers to the appropriate use of the potties. Participants believed that potty training was more difficult in older children (more than 2 years) compared to younger ones (under 1 year), reflecting a common perception that introducing potty training when children are very young would be more effective (approximately 6 months of age).

One of the participants shared her experience in trying to teach her older child to use the potty:

> *"I tried in many ways to help my child (36 months) to defaecate in the potty following the strategies the health promoters discussed. When he gave a signal of defaecation, I let him sit on the potty but he would not defaecate."*

Participants also mentioned that it was the role of the mother to potty train and dispose of the child's faeces. If the mother was engaged in other household tasks or was absent when the defaecation event occurred, faeces disposal was delayed.

> *"Everybody loves a child but nobody shares the task of faeces collection and disposal. I have to do this myself."*

If a child defaecated in a neighbouring compound, the child's mother was called to dispose of the faeces. Participants stated that until the mother removed the faeces, it was usually left there. One participant specified,

> *"We call the child's mother (whose child had defaecated) and she removes the faeces."*

Other difficulties in keeping the household environment free of faeces were the constant presence of poultry and other domestic animals that regularly produced faeces and the use of cow dung as bio-fuel. Participants also found it difficult to remove loose, liquid faeces from uneven or hard surfaces with the sani-scoop. The perceptions that the faeces of one's own child and some animal faeces are not disgusting also may have limited the motivation to dispose of faeces.

*"Cow dung is not disgusting to us (the farmers), it's our resource. We use cow dung in our field as manure to make our land fertile to increase productivity."*

In addition, household members are not likely to change the current practice of removing small animal faeces by sweeping the courtyard twice a day even though poultry produce faeces several times throughout the day.

*"We sweep our courtyard twice a day. We sweep the dirt courtyard after we wake up in the morning and remove the ashes from the chula (mud stove). Then we sweep our houses and the leaves that drop during the night. In the evening we sweep the courtyard again and remove any poultry droppings because it is uncomfortable to walk through them."*

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***Comments***

Although households reported high rates of hardware use at three months, we did not observe a change in the presence of faeces in the household environment in this study. A qualitative study to investigate the underlying reasons for the lack of change in behaviour found that insufficient potty training was a barrier to the effective and consistent use of potties (9). Inconsistent potty use resulted in children defaecating in the courtyard and delayed disposal of faeces from the household environment.

The presence of child and animal faeces even after high reported use of distributed hardware may also have occurred for practical reasons. Potty training is a long process and a new social behaviour in rural Bangladesh. A three-month intervention period may have been too short to have produced any meaningful results. Furthermore, the variation in children's ages (6-36 months) may have also affected the successful initiation of potty training. The sani-scoop also has its limitations when used to remove small faeces like poultry droppings, watery faeces, and when removing faeces from hard surfaces like coated household floors.

Furthermore, since we used observed faeces as a crude measurement of environmental contamination, it is possible that the data collected were affected by variability in defaecation behaviours, such as the typical time of day when children defaecate, or by seasonality, as faeces might be more likely to be washed away during the rainy season. Nevertheless, this indicator can be a useful measure of open defaecation and is relatively simple to record. In this study, it was an important measure that helped us recognize that while hardware appeared popular, its use was limited, and this led us to undertake a study to investigate reasons for suboptimal use of the hardware.

Future sanitation interventions that include potties and sani-scoops should involve secondary caregivers in addition to mothers during the potty

training process to encourage consistent use and adequate disposal of faeces. When mothers are busy, sick, or absent from the household, a secondary caregiver may be able to assist the child to ensure consistent potty use. Domestic animal faeces, such as cow dung, are a part of rural livelihoods and are perceived as useful. Thus, interventions should not aim to completely remove all domestic animal faeces from rural household environments, but should aim to contain all faeces at a safe distance to prevent direct exposure of children to them. Considering the high prevalence of animal faeces in rural household environments, more focus should be placed on hygienic disposal of child rather than animal faeces. Although promoting the safe disposal of child faeces is often a part of sanitation interventions, additional research is needed to develop practical strategies to reduce the contamination of a child's household environment to optimize the impact of the intervention.

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# Unusual waterfowl mortality due to highly pathogenic avian influenza A (H5N1) virus in Netrokona, Bangladesh, 2011

**We investigated reports of unusual illness and death among ducks and geese with suspected highly pathogenic avian influenza A (H5N1) (HPAI H5N1) virus infection in northeast Bangladesh by conducting door-to-door visits in six affected communities to identify the cause and extent of the outbreak and to sample sick and dead poultry. We also conducted a community survey to identify suspected human influenza-like illness cases. In the affected communities, 61% (1,789/2,930) of all the chickens, 36% (1,425/3,929) of all the ducks and 83% (220/265) of all the geese died within the 14 days preceding the investigation. Of the 72 poultry sampled, 78% (n=56) were positive for HPAI H5N1. Among the 10 human cases showing symptoms compatible with influenza illness, four were positive for influenza A/H3 and none were positive for HPAI H5N1. The pandemic potential of HPAI H5N1 and its high mortality rate in domestic poultry warrant continued surveillance and a comprehensive prevention and control programme.**

Highly pathogenic avian influenza A (H5N1) (HPAI H5N1) virus continues to be a major concern worldwide because of its ability to cause significant morbidity and mortality in birds, its ability to infect humans, and its pandemic potential (1). Wild aquatic birds are natural reservoirs of avian influenza (type A) viruses and play an important role in the ecology and propagation of the virus (2).

Although mortality due to HPAI H5N1 is high (75-100%) in domestic chickens (3), it does not typically cause clinical disease or death in ducks or wild aquatic birds. However, since 2002, HPAI H5N1 has caused several sporadic outbreaks in ducks and geese (4,5). Recent outbreaks due to HPAI H5N1 in ducks and geese have been reported in India, in 2011, and Indonesia, in 2012-13 (6,7). Since 2007, there have been 545 reported HPAI H5N1 outbreaks in chickens in Bangladesh, causing devastating financial loss in the poultry industry (8). However there have not been any previously reported deaths in domestic ducks or geese (9). As of March 2013, six human cases of HPAI H5N1 infection have been identified in Bangladesh and all had exposure to sick poultry (10). The detection of avian influenza in humans illustrates the ongoing risk of human infection with novel influenza strains.

Since 2009, the Institute of Epidemiology, Disease Control and Research (IEDCR) has monitored unusual health and health-related outcomes in humans and in animals associated with human cases reported in print and

electronic media to facilitate rapid response to possible outbreaks of disease. In June 2011, this event-based surveillance identified a local newspaper report about unusual duck and goose illness and deaths in backyards and in small commercial duck and goose farms in Netrokona, a northeastern district of Bangladesh. The affected birds demonstrated neurological signs such as twisting of the head and neck, uncoordinated movements and head tilting; these signs are infrequently observed among sick poultry.

Upon identification of the report, IEDCR, Department of Livestock Services and icddr,b formed a collaborative team of epidemiologists, physicians and veterinarians to conduct an outbreak investigation. The objective of this investigation was to understand the cause and the extent of duck and goose mortality and to identify any possible associated human infections.

In collaboration with the district livestock officer, we visited the outbreak sites (Figure 1) and prepared a line list of affected flocks. We defined a case of unusual poultry mortality as >10% poultry mortality in a flock in which affected birds showed twisting of their heads and/or necks. We conducted door-to-door visits to households in the affected communities to identify any poultry that had become sick and died in the 14 days preceding the investigation. The survey team collected data from each household on flock sizes, dates of onset of illness, and clinical signs in poultry. We collected oropharyngeal and cloacal swab samples from each flock of ducks or geese that met the case definition. We also performed a post mortem examination on the ducks, geese, and chickens to record gross lesions. Swabs were tested for HPAI H5N1 virus in icddr,b's animal laboratory by real time reverse transcriptase-PCR (rtRT-PCR). Samples were also sent to the Influenza Division of the Centers for Disease Control and Prevention, USA where virus isolation was performed and isolates were further sequenced and characterized.

During the outbreak investigation, the field team also conducted a community survey to identify cases of influenza-like illness (ILI). The team enlisted the persons who met the ILI case definition (subjective fever and either cough or sore throat) and whose onset of symptoms was during the investigation period. Study physicians interviewed ILI cases, recorded their demographic, clinical and exposure history and collected naso-pharyngeal and throat swabs for testing for influenza virus. The specimens were tested for influenza A and B viruses and influenza A virus-positive samples were further subtyped for A/H5, A/H1 and A/H3 virus by rtRT-PCR at icddr,b.

We identified six communities with unusual mortality among ducks and/or geese and surveyed 300 flocks of which 90% (n=270) had experienced at least one poultry death. In the 14 days preceding the investigation, 61% (1,789/2,930) of all the chickens, 36% (1,425/3,929) of all the ducks and 83% (220/265) of all the geese in the six identified communities died (Figure 2).

*Figure 1: Location of communities affected with highly pathogenic avian influenza (HPAI H5N1) and dates of onset of illness in ducks and geese in sub-districts of Netrokona District of Bangladesh, June-July 2011*

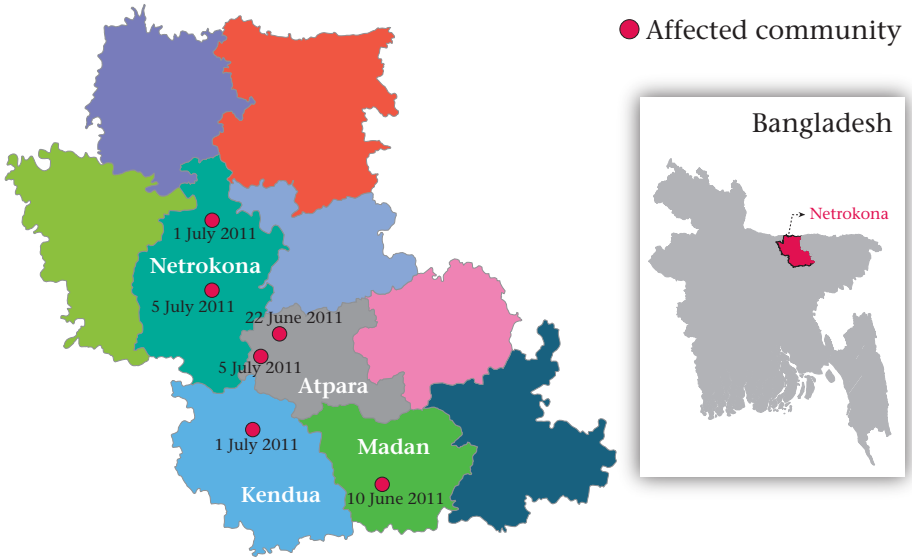
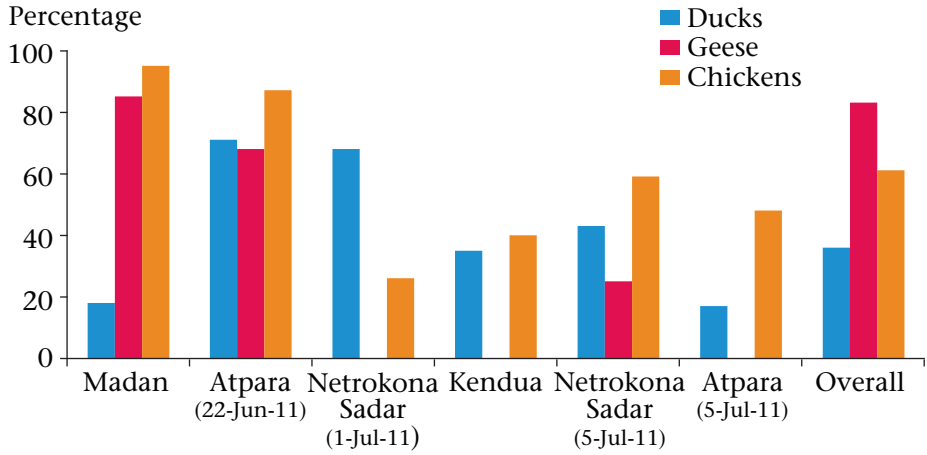



*Figure 2: Poultry mortality observed in different sub-districts of Netrokona District, Bangladesh, June-July 2011*




The common characteristics among the duck flocks were twisting of the head and neck, whitish watery diarrhoea, uncoordinated movements, lack of appetite and sudden death. The geese exhibited tilting of the head and neck, uncoordinated movements, leg paralysis, and sudden death. Chickens had

cyanosis of the comb and wattle, leg hemorrhage, drowsiness and sudden death. The internal organs appeared normal except for mild congestion and pin point hemorrhages noted in the liver and pancreas and evidence of air sac infection in ducks, geese, and chickens.

Of the 72 poultry sampled, 78% (n=56) of the birds were positive for HPAI H5N1 by RT-PCR, including 91% (49/54) of ducks, 38% (3/8) of geese and 40% (4/10) of chickens. Six viruses were isolated and further characterized. Phylogenetic analysis showed that the isolates were all clustered within a sub-lineage of HPAI H5N1 virus (clade 2.3.2.1), indicating that the outbreak was caused by a single lineage of HPAI H5N1.

Among the 10 identified human cases of ILI, three were from the same household. The mean age of ILI cases was 12 (range 8-16) years. All the case-patients had fever, cough, and runny nose and only one case-patient did not have a sore throat. Of the 10 persons we interviewed, nine had exposure to poultry in their houses, six had held poultry in their bare hands, four assisted in poultry slaughtering, and two visited a live bird market in the 14 days preceding the onset of illness. Swabs from four (40%) of the persons with ILI tested positive for influenza A/H3, however all swabs were negative for influenza B, influenza A/H1 and A/H5.

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***Comments***

Clinical manifestations, laboratory tests, and epidemiological data from this investigation indicate that the cause of the morbidity and mortality in ducks, geese and chickens was due to HPAI H5N1. HPAI H5N1 viruses are usually highly pathogenic in chickens, but do not typically cause clinical disease in ducks or wild aquatic birds (11). However, since 2002, a number of HPAI H5N1 viruses have been shown to cause clinical signs and mortality in ducks either by natural infection or in experimental studies (4,5). Recently, there have been several outbreaks in domestic ducks and/or geese mortality reported in India, Egypt, China, and Indonesia, due to HPAI H5N1 (6,7,12,13). In the past, HPAI H5N1 outbreaks among ducks and geese had been reported sporadically from several countries of Asia, Africa and Europe (4,5,12). The potential for HPAI H5N1 to cause morbidity and mortality in domestic ducks and geese is a serious problem for poultry raisers in Bangladesh that have already been affected by HPAI H5N1 among chickens, because poultry are an important source of income and nutrition for them. Additionally, any geographical spread of HPAI H5N1 in poultry

could result in further human exposure to this virus.

In this outbreak, HPAI H5N1 was not isolated from humans, indicating that there was no detectable transmission of the virus from poultry to humans. The findings of influenza A/H3 among the poultry raisers are congruent with findings from hospital-based influenza surveillance indicating circulation of seasonal influenza among humans in Bangladesh at the time of the outbreak. However, the simultaneous circulation of seasonal influenza in humans and HPAI H5N1 in birds in the same locality is a concern as this could increase the chances of reassortment between human-adapted influenza strains and HPAI H5N1.

This outbreak of morbidity and mortality in ducks and geese was due to HPAI H5N1. Although HPAI H5N1 was not identified in humans, the potential for human infection and its high mortality rate in domestic poultry warrant enhanced surveillance efforts to facilitate disease control and prevention programmes and support the need for pandemic preparedness. We recommend that any unusual outbreak among poultry, including waterfowl, be immediately reported to the appropriate local veterinary and health authorities. Reports of unusual illness in poultry or waterfowl should be shared with clinicians practicing in the affected region so they may have heightened awareness of respiratory illness in their patients, particularly those with exposure to poultry and waterfowl.

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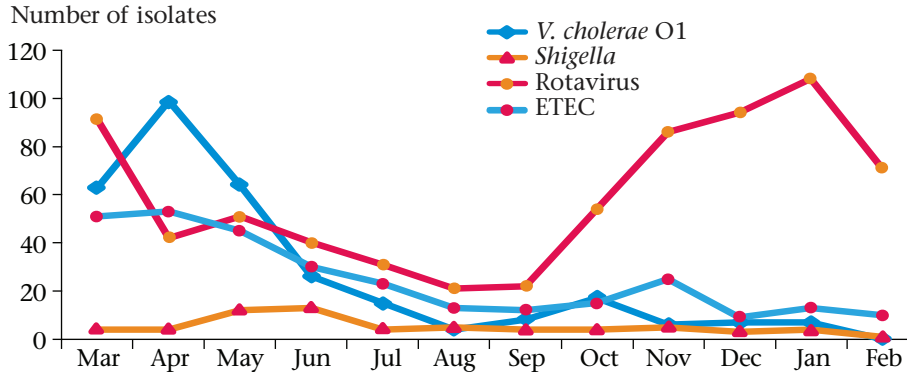
# Surveillance updates

With each issue of HSB, updates of surveillance data described in earlier issues are provided. These updated tables and figures represent the most recent observation period available at the time of publication. We hope these updates will be helpful to health professionals who are interested in current patterns of disease and drug resistance in Bangladesh.

***Proportion of diarrhoeal pathogens susceptible to antimicrobial drugs: March 2012-February 2013***

| Antimicrobial agents | *Shigella* (n=63) | *V. cholerae* O1 (n=316) |
|---|---|---|
| Nalidixic acid | Not tested | Not tested |
| Mecillinam | 88.9 | Not tested |
| Ampicillin | 55.6 | Not tested |
| TMP-SMX | 25.4 | 1.3 |
| Ciprofloxacin | 55.6 | 100.0 |
| Tetracycline | Not tested | 4.4 |
| Azithromycin | 77.4 | 99.7 |
| Ceftrioxone | 98.4 | Not tested |

***Monthly isolation of* V. cholerae *O1,* Shigella, *Rotavirus and ETEC: March 2012-February 2013***

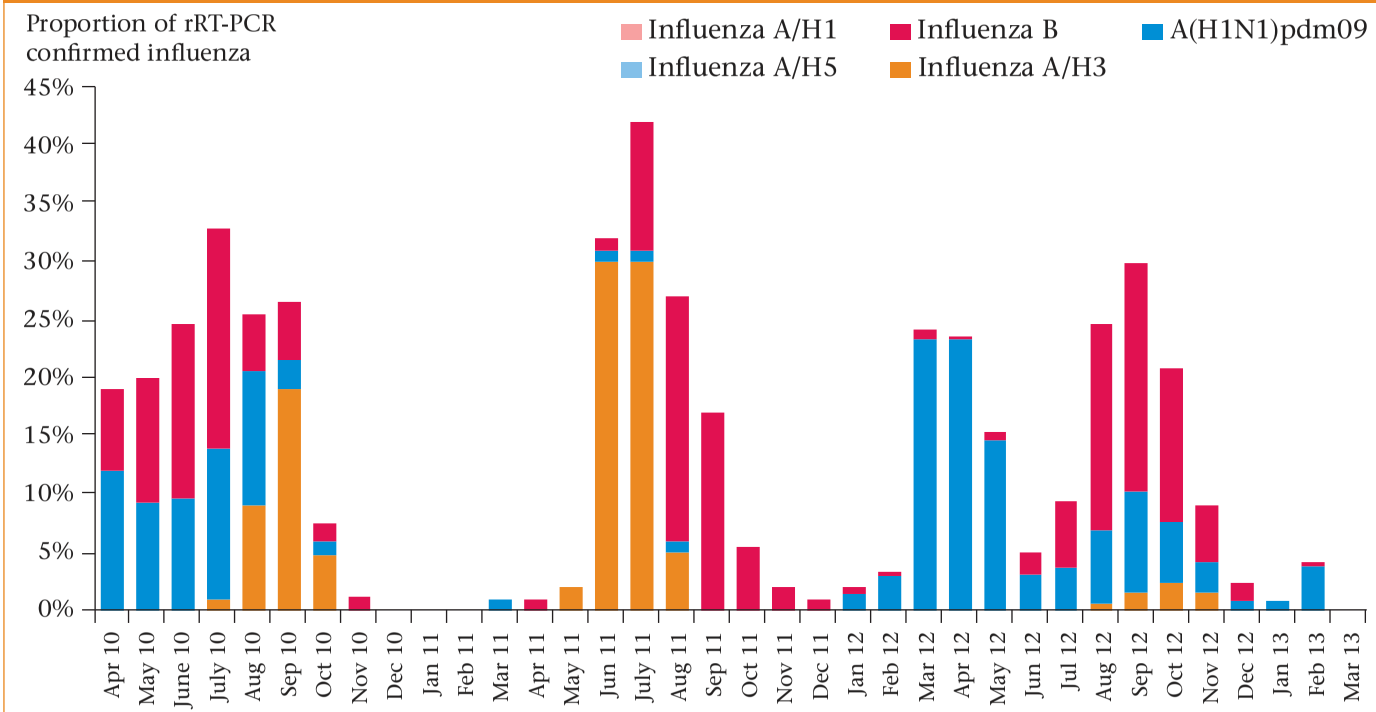



***Antimicrobial susceptibility pattern of* S. typhi *among children <5 years during January-March 2013***

| Antimicrobial agent | Total tested (n) | Susceptible n (%) | Reduced susceptibility n (%) | Resistant n (%) |
|---|---|---|---|---|
| Ampicilin | 17 | 14 (82.0) | 0 (0.0) | 3 (18.0) |
| Cotrimoxazole | 17 | 14 (82.0) | 0 (0.0) | 3 (18.0) |
| Chloramphenicol | 17 | 13 (78.0) | 0 (0.0) | 4 (24.0) |
| Ceftriaxone | 17 | 17 (100.0) | 0 (0.0) | 0 (0.0) |
| Ciprofloxacin | 17 | 0 (0.0) | 17 (100.0) | 0 (0.0) |
| Nalidixic Acid | 17 | 0 (0.0) | 0 (0.0) | 17 (100.0) |

Source: icddr,b's urban surveillance in Kamalapur (Dhaka).

***Proportion of laboratory confirmed influenza among hospitalized severe acute respiratory illness (SARI) and outpatient influenza like illness (ILI) cases between April 2010 and March 2013***




Source: Patients participating in hospital-based influenza surveillance in Dhaka National Medical College Hospital, Community-based Medical College Hospital (Mymensingh), Jahurul Islam Medical College Hospital (Kishoregonj), Rajshahi Medical College Hospital, Shaheed Ziaur Rahman Medical College Hospital (Bogra), LAMB Hospital (Dinajpur), Bangabandhu Memorial Hospital (Chittagong), Comilla Medical College Hospital, Khulna Medical College Hospital, Jessore General Hospital, Jalalabad Ragib-Rabeya Medical College Hospital (Sylhet) and Sher-e-Bangla Medical College Hospital (Barisal)

**Twisting of head and neck observed in a duck in June-July 2011, Netrokona, Bangladesh**

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