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Bangladesh anthrax outbreaks are probably caused by contaminated livestock feed

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SUMMARY

In Bangladesh from 1 July to 30 September 2010 there were 104 animal cases of anthrax and 607 associated human cases. This investigation was conducted in Sirajganj district in December 2010, on eight farms where animal cases had occurred. *Bacillus anthracis* was recovered from soil samples and turbinate bones on six farms. Canonical single nucleotide polymorphism (SNP) analysis showed that all the isolates belonged to the major lineage A, sublineage A.Br.001/002 of China and South East Asia while a multilocus variable-number tandem-repeat (VNTR) analysis (MLVA) with 15 VNTRs demonstrated three unique genotypes. The single nucleotide repeat (SNR) analyses showed two SNR types in 97 out of 99 isolates; nevertheless, due to its higher discriminatory power the presence of two isolates with different SNR-type polymorphisms were detected within two MLVA genotypes. The epidemic occurred during the monsoon season, a time of extensive flooding, suggesting that the source was contaminated feed, not grazing, which is supported by the genetic variance.

Key words: Anthrax.

INTRODUCTION

In Bangladesh, anthrax is considered an endemic disease affecting humans and ruminants. The Department of Livestock (DLS) registered 437 animal cases in 2008 and 449 animal anthrax cases in 2009. In 2010 DLS registered 104 animal cases in the 3 months from 1 July to 30 September 2010. For the period 18 August to 2 October 2010, the Institute of Epidemiology, Disease Control & Research (IEDCR) reported 607 human anthrax cases in twelve districts – Chittagong, Kushtia, Lalmanirhat, Lakshmipur, Meherpur, Manikganj, Narayangonj, Pabna, Rajshahi, Satkhira, Sirajganj,

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Table 1. *2010 monsoon bovine diets on the eight farms visited*

	Kamarkhand				Shahjadpur			
					Chitulia			
Feed	NIM	MH	Belkuchi OM	Ullapara HM	SA	AA	NI	Khamar Shanila AR
Rice straw	+	.	+	+	+	+	+	+
Broken rice	+	.	+	.	.	.	.	.
Rice bran	.	.	+	.	.	.	.	.
Wheat bran	+	.	+	+	+	+	+	+
Water hyacinth	.	+	.	.	.	.	.	.
Roadside (Napier grass)	.	.	.	+	.	.	.	.
Cut grass (ex-field)	.	.	.	.	.	.	.	+
'Island' grazing	.	.	+	.	.	.	.	.
Pelleted feed	.	.	.	.	.	.	+	.
Mustard oil cake	+	.	+	+	.	.	.	.
Sesame oil cake	.	.	.	.	+	.	+	+

and Tangail [1]; virtually all were cutaneous and there were no human deaths. Overall, anthrax was reported in humans and/or livestock in 15 districts. A collaborative investigation team from IEDCR and the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B) interviewed 202 people who had been affected during 23 July to 22 September [2]. These people were affected after they came into contact with contaminated carcases and/or the butchered products thereof, e.g. blood during slaughtering, raw meat. Generally, the owners of the animals became upset when they observed their animals in a moribund condition. This encouraged them to slaughter and butcher their animals and sell the meat to recover some money. However, a few owners did not slaughter their sick animals because they believed that slaughtering sick, pregnant or under-aged animals is forbidden by their Muslim religion. Moreover, some sick stock died before the owner could organize slaughter and butchering. Usually, 10–15 people were involved in the process of slaughtering, butchering, and the distribution of the meat, and community members were invited to buy the meat at a reduced price. Animals found dead were usually abandoned outside the farm compound. Sometimes dead animals were dragged some distance out into the rice fields and left in a marginal place. Custom allows the low-caste Hindu *Muchi* to skin abandoned animal carcases; they process and sell hides, or make and repair shoes from them.

The 2010 series of anthrax outbreaks occurred during the monsoon season when the affected area of Bangladesh is flooded and any potential grazing is covered by water.

MATERIALS AND METHODS

Field visits and sampling

This investigation over 2 weeks in December 2010, was conducted in the Sirajganj district, which can be considered the centre of the epidemic, because 219 (36%) human cases were registered in that district in 2010, more than twice the number in any other district. Visits were made to farms in the following subdistricts: two farms (NIM, MH) in Kamarkhand, Dhopakali village; one farm (OM) in Belkuchi, Adachaki village; one farm (HM) in Ullapara; four farms in Shahjadpur with three (SA, AA, NI) in Chitulia village, and one farm (AR) in Khamar Shanila village; and the farmers were interviewed regarding the events during each outbreak and what their livestock had been fed at the time (see Table 1 for details). Animal and human anthrax cases were registered during 2010 on all these farms. Soil samples, about 50 g each, were collected from suspected contaminated sites within each farm compound, such as where sick animals had been slaughtered and butchered or had been buried. As three skulls were still available on one farm, turbinate bones were recovered from each, and individually bagged.

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Isolation

The ground anthrax Bacillus refined identification (GABRI) method was used to recover *Bacillus anthracis* organisms from the soil samples. This test, developed in the laboratories of the Anthrax Reference Institute of Italy in Foggia, is able to culture *B. anthracis* in contaminated soil which contain some 200 spores in 7·5 g soil (data not shown) or at a threshold value of 27 spores/g soil. Briefly, 7·5 g soil were added to 22·5 ml washing buffer (sterile distilled water solution containing 0·5% Tween 20) and shaken for 30 min. The solution was then centrifuged at 657 ***g*** for 5 min and the supernatant was incubated at 54 °C for 20 min. After this incubation, 3 ml supernatant was mixed with 3 ml phosphomivin tryptose soya broth (PTSB), and 1 ml of the supernatant/PTSB was sown onto a plate of trimethoprim, sulfamethoxazole, polymixine 5% sheep blood (TSMP) agar. The plates were incubated aerobically at 37 °C for 24 h. Suspected colonies on the TSMP plates were identified by colony morphology and Gram staining. All tests were run in the Institute's BL3 security laboratory.

DNA preparation and PCR

Each *B. anthracis* suspected colony was streaked onto 5% sheep blood agar plates and then incubated at 37 °C for 24 h. After heat inactivation (98 °C for 20 min), microbial DNA was extracted using the DNAeasy Blood and Tissue kits (Qiagen, USA), following the protocol for Gram-positive bacteria. Specific PCR assays were used to confirm *B. anthracis* [3].

Canonical single nucleotide polymorphism (SNP) analysis

We utilized 13 TaqMan-Minor Groove Binding (MGB) allelic discrimination assays with oligonucleotides and probes as described by Van Ert *et al.* [4] for each of 13 canonical SNPs. Each 5 μl reaction contained 1 × TaqMan Genotyping Master Mix (Applied Biosystems, USA), 250 nM of each probe, and 600 nM each of forward and reverse primers and 1·0 μl of ~1 ng template DNA. For all assays, thermal cycling parameters were, 95 °C for 10 min, followed by 50 cycles at 95 °C for 15 s and 60 °C for 1 min. Endpoint fluorescent data were measured on the ABI 7900HT. Canonical SNP profiles were compared to the 12 recognized worldwide sublineages and subgroups [4].

15-loci multilocus variable-number tandem-repeat (VNTR) analysis (MLVA) and single nucleotide repeat (SNR) analyses

To obtain higher genetic differentiation in very closely related isolates, VNTR loci were investigated, paired with SNRs loci that are molecular markers with extreme discriminatory power. We utilized 5'-fluorescent-labelled oligonucleotides, deprotected and desalted, specifically selected for the VNTRs and SNRs used. The 15 specific primer pairs for the MLVA were selected as described by Van Ert *et al.* [4]. The four specific primer pairs for SNR reactions were selected according to Garofolo *et al.* [5]. MLVA polymerase chain reactions (PCRs) were performed in two multiplex reactions in a final volume of 15 μl. The reaction mixture contained: 1 × PCR reaction buffer (Qiagen), 1 U of HotStarTaq Plus DNA polymerase (Qiagen); dNTPs (0·2 mM each); 3 mM $MgCl_2$ and appropriate concentrations of each primer (multiplex 1: vntr12, 0·37 μM; vntr35, 0·37 μM; vntr23, 0·2 μM; vntr16, 0·2 μM; vntr17, 0·2 μM; vrrB2, 0·2 μM; vrrC1, 0·1 μM; vntr32, 0·1 μM; multiplex 2: vntr19, 0·2 μM; CG3, 0·2 μM; vrrB1, 0·2 μM; pX01, 0·2 μM; pX02, 0·2 μM; vrrA, 0·1 μM; vrrC2, 0·1 μM); and 1 ng template DNA. The thermocycling conditions were as follows: 95 °C for 3 min; 35 cycles at 94 °C for 30 s, at 60 °C for 45 s, and at 72 °C for 1 min, and finally, 72 °C for 5 min.

The SNR PCR was performed in a multiplex reaction in a final volume of 15 μl containing 1 × PCR reaction buffer (Qiagen); 1 U HotStarTaq Plus DNA polymerase (Qiagen); dNTPs (0·2 mM each); 3·5 mM $MgCl_2$, dNTPs; and appropriate concentrations of forward and reverse primers (HM1, 0·2 μM; HM2, 0·2 μM; HM6, 0·2 μM; HM13, 0·1 μM); and 1 ng template DNA. The thermocycling conditions were as follows: 95 °C for 5 min; 35 cycles at 94 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s, and finally, 72 °C for 5 min.

Automated genotype analysis

The MLVA PCR products were diluted 1:80 and subjected to capillary electrophoresis on an ABI Prism 3130 genetic analyzer (Applied Biosystems Inc.) with 0·25 μl GeneScan 1200 and sized by GeneMapper 4.0 (Applied Biosystems Inc.) Amplified SNR PCR products were diluted 1:80 and subjected to capillary electrophoresis on ABI Prism 3130 genetic analyzer (Applied Biosystems) with 0·25 μl GeneScan 120 LIZ, and sized by GeneMapper 4.0

Table 2. *Farms visited in Sirajganj district and* Bacillus anthracis *recovered*

Subdistrict	Farm	Site where sample obtained	Sample ID	No. of isolates
Kamarkhand	NIM	Burial site	1	4
	NIM	Burial site	2	4
	MH	Burial site	3	5
	MH	Burial site	4	5
	MH	Burial site	5	10
Belkuchi	OM	Bleeding site	6	5
	OM	Bleeding site	7	5
	OM	Skinning site	8	5
	OM	Burial site	9	5
Ullapara	HM	Burial site	10	5
	HM	Burial site	11	5
Shahjadpur	SA	Barn site	12	7
	SA	Barn site	13	1
	AA	Burial site	14	0
	AA	Burial site	15	0
	NI	Burial site	16	0
	NI	Burial site	17	0
	AR	Turbinate, wet skull	18	12
	AR	Turbinate, dry skull A	19	1
	AR	Turbinate, dry skull B	20	20

(Applied Biosystems Inc.). In all the analyses the samples were processed in triplicate and the concordance of the results allowed the correct sizing of the fragments.

GIS development and outbreak mapping

A GIS database was constructed from GPS coordinate pairs (latitude/longitude) of farm locations where soils were collected during this investigation. Map symbology was derived from the MLVA-15 and SNR analysis to map the geographical distribution of genotypes and SNR polymorphisms. We also mapped the districts that reported human cases using administrative boundary data from the Global Administrative Areas website (www.gadm.org). All GIS analyses were performed in ArcGIS 10 (ESRI, USA).

RESULTS

Farm interviews

In the areas we visited the farmsteads and narrow rural roads are constructed from the inceptisol clay soil from nearby borrow pits and are raised some 1·5–3 m above the surrounding paddy fields. The housing for dairy cattle is modest and may or may not have a loose brick floor; brickworks are a common feature in the region. The numbers of cattle we saw on the eight farms visited varied from three to 11 head. The cattle were held in the farm compound and largely handfed during the monsoon season – the time of the epidemic – and therefore could have been infected by one of the feeds reported to us by the owners; traditional infection through grazing would not have occurred as the fields are flooded during the monsoon and only one herd had access to any grazing. The farmer on OM had grazed his livestock on an 'island' of clay soil for a short time during the day before bringing them back each evening to be fed with rice straw, wheat bran, rice bran, and mustard oil cake. Three herds (from farms HM, NI, SA) had not grazed in the 1–2 months before cases occurred. Table 1 lists the various feeds the cattle received on the eight farms during that time, as reported by the farmowners. Two herds were fed cut forage and the cut was 7–10 cm off the ground. While 6/8 herds were fed mustard and sesame oil cakes, two herds did not receive either of these. The farmer on MH only fed his stock water hyacinth. However, his farm was close to farm NIM where a cow had died, and was buried in the compound. Farm AA was close to farm SA and two of farm AA's animals died a 'few' days (the interval is unclear) after farm SA had lost four goats, three sheep, and a cow and a calf, which would have

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Table 3. *Presence of three novel genotypes based on MLVA with 15 VNTRs*

Area	VNTRs													MLVA type
	pX01	pX02	cg3	vrra	vrrb1	vrrb2	vrrc1	vrrc2	vntr12	vntr16	vntr17	vntr19	vntr23	vntr32	vntr35	
Khamar Shanila	127	133	153	295	223	154	584	522	110	135	381	90	182	563	***109***	Gt/Kha
Chitulia, Ullapara	127	133	153	295	223	154	584	522	110	135	381	90	182	563	116	Gt/ChU
Kamarkhand & Belkuchi	127	133	153	***306***	223	154	584	522	110	135	381	90	182	***378***	116	Gt/KamBel

MLVA, Multilocus variable number tandem repeat (VNTR) analysis.

presented ample opportunities for haematophagous flies to spread infection to nearby livestock.

B. anthracis *recoveries*

B. anthracis was isolated from 16/20 samples, for a total of 99 isolates (Table 2). A quantitative analysis of two samples, nos. 1 and 5, showed a high concentration of some 2×10^6 spores/g soil.

Genomic analysis

The canonical SNP analysis showed that all the isolates belonged to the major lineage A, sublineage A.Br.001/002 according to the worldwide distribution of *B. anthracis* clonal lineages [4].

The MLVA on 15 VNTRs on the isolates showed the presence of three different novel genotypes, which we have labelled Gt/Kha, Gt/ChU, and Gt/KamBel (Table 3).

The SNR analysis showed the presence of two SNR types (SubGt-1, SubGt-2) in 97/99 isolates and was able to detect the presence of two isolates with different SNR-type polymorphisms (SubGt-1a, SubGt-2a) within the MLVA genotypes Gt/Kha and Gt/KamBel (Table 4). Moreover, two samples yielded two different isolates each: bleeding site soil sample (no. 6) from farm OM provided four isolates of SubGt-2 and one SubGt-2a; the wet skull at Khamar Shanila had 11 isolates of SubGt-1 and one of SubGt-1a.

Figure 1*a* illustrates the districts reporting human cases during the 2010 outbreak period and Figure 1*b* illustrates the geographical locations of the eight farms sampled during this investigation, and the associated genotypes and SNR polymorphisms.

CONCLUSIONS

This 2010 epidemic in Bangladesh demonstrated that anthrax has the potential to be a serious zoonotic disease in low-income countries where there are few resources for an optimal infectious diseases control system in humans or livestock. It underscored something long known about this disease – the high risk of human exposure when slaughtering and butchering infected animals. The raw meat, skins, and blood of infected animals are dangerous vehicles of infection for humans.

The MLVA analysis of the isolates demonstrated the presence of three genotypes. As noted previously

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Table 4. *SNR analyses*

MLVA type	SNR type	No. isolates	SNRs			
			HM1	HM2	HM6	HM13
Gt/Kha	SubGT-1	32	79	108	91	118
Gt/Kha	SubGT-1a	1	***80***	108	91	118
Gt/ChU	SubGT-1	18	79	108	91	118
Gt/KamBel	SubGt-2	47	83	110	90	118
Gt/KamBel	SubGt-2a	1	83	110	***91***	118

SNR, Single nucleotide repeat; MLVA, multilocus variable number tandem repeat (VNTR) analysis.

the Chitulia and Ullapara outbreaks had the same genotype named Gt/ChU, which is different from the Khamar Shanila genotype, Gt/Kha, in the size of vntr35, and from the genotype of Gt/KamBel in relation to vrra and vntr32. These limited samples suggest that the genotypes are specific for each territory but it should be noted that these were only six farms out of the many affected in 2010. Our results show that SNR analysis, although analysing hypervariable loci, demonstrated a low discriminatory power among all the isolates with only two SNR types detected, but at the same time the anaysis was able to detect two isolates with different SNR genotypes. These findings confirm the ability of SNRs to mutate rapidly, suggesting the presence of mutational steps due to multiple replication.

The SNP analysis demonstrated that all these genotypes belong to the A.Br.001/002 sublineage. This result indicates that some of the *B. anthracis* strains circulating in Bangladesh are related to the strains circulating in China and other South West Asian countries. This is in contrast to the historical and economic interactions between India and Bangladesh. Van Ert *et al*. [4] indicated that in India the predominant linage is A.Br.Austr.94 which is different from the lineage present in Bangladesh. However, it should be noted that very few isolates of Indian *B. anthracis* have been genomically studied; it is truly a genomic *terra incognita*.

One of the singular characteristics of animal feed-based outbreaks of bovine anthrax is the multiplicity of genomic strains that are recovered. Different strains are found in different herds in the same epidemic, although they have a common merchantile source; also different strains from different cows in the same herd, and occasionally multiple strains will be recovered from individual animals. This occurs due to the mixing of bones from many sources in the commercial production of bonemeal, which is historically the commonest source of infection in livestock feed. On the other hand epidemics following from sporadic grazing cases multiplied by the activities of haematophagous flies largely involve a singular strain in herd after herd and markedly reduce any regional genomic diversity, see for example Smith *et al*. [6]; occasionally a second or third strain may be found but their frequency is around 1% of affected herds and reflects coincident but parallel series of outbreaks [7]. To have found such a diversity of MLVA-type strains ($n=3$) in the samples recovered from just six geographically close herds (see Fig. 1) strongly supports the hypothesis of contaminated feed as the source, the nature of which has yet to be determined. This is reinforced by finding two dual SNR infections, one from some bloody soil and the other from turbinate bones months after the animals died. However, in regard to these dual infections it should be borne in mind that these sites are hypervariable and thus the duality might be coincidental.

While wheat bran might appear to be a possible source, it should be noted that bran is a by-product of milling any grain, and should not be confused with chaff, which is the coarse, scaly material surrounding the grain but does not form part of the grain itself. Bran is the hard outer layer of any grain and of distinct dietary advantage being rich in dietary fibre and essential fatty acids and significant quantities of starch, protein, vitamins and minerals. But if the seed heads were to become contaminated, which is highly unlikely being some distance from the soil, it would be the outer scaly material that would be contaminated,

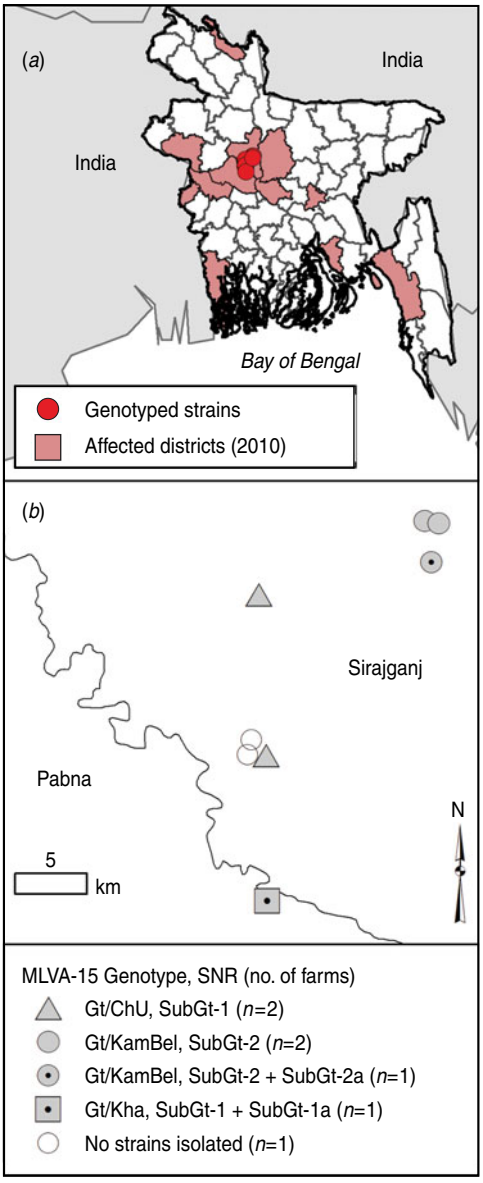

Fig. 1 [*colour online*]. (*a*) The geographical distribution of districts reporting human cases in 2010 (■) and the location of the eight farms sampled during this investigation (●). (*b*) For the eight farms, grey symbols represent the three MVLA-15 defined genotypes. Grey symbols with black dots represent SNR polymorphisms. Open circles indicate sample locations where no strains were recovered.

not the grains. Further, the later processing of the bran would dilute the effect of spores. The most probable source would be the oil cakes as the initial mustard and sesame seed residues as a livestock feed are supplemented with bonemeal for the calcium to maximize milk production as well as essential minerals such as copper, selenium, and zinc. The composition of these oil cakes is left for later investigation and their contribution to this disease to be confirmed by laboratory analyses. As a result of the informed outbreak investigations by ICDDR,B during 2011 various samples were collected, including livestock feeds. These samples were sent to the senior author (A.F.) for analysis; a number of the samples, not otherwise specified, were found to contain *B. anthracis*, confirming our earlier suspicions. The specifics of this feed contamination must await ICDDR,B investigations.

Pabna and Sirajganj, two adjacent districts with some 47% of reported human anthrax cases in 2010 [1], have the highest cattle densities in Bangladesh and are also the two main milk-producing districts in the country [8]. Efficient production of milk demands feed supplements. The in-country handling of livestock found dead and the possibility of domestic recycling of bone as bonemeal to meet the livestock feed demand raises the hypothetical possibility that this could be an intra-national Bangladeshi problem. This could explain the repeated outbreaks year after year in a region with clay soils, which normally do not support anthrax spore survival [9] and over which monsoon floods would regularly flush away any surface deposited spores, and any carcases buried in the field would be sealed in by the clay. It is also possible that contaminated bonemeal or other contaminated livestock feed products may be being imported from neighbouring countries, including China and other South West Asian countries. Unfortunately the genomic characterstics of *B. anthracis* in neighbouring West Bengal and Myanmar are unavailable. If contaminated bonemeal is confirmed as the source of infection it would radically simplify control and surveillance for testing and certifying that all commercial livestock feed additives, especially bonemeal, whether local or imported, do not contain spores of *B. anthracis*. Improving the quality of animal feed may prove to be cheaper, faster, and more cost-effective than livestock vaccination, which to date has had limited success in preventing livestock anthrax in Bangladesh.

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DECLARATION OF INTEREST

None.

REFERENCES

1. **Institute of Epidemiology, Disease Control & Research (IEDCR).** Anthrax update, 2010 (http:www.iedcr.org/Healthmessages/Anthrax_Update_last.pdf). Accessed 25 April 2011.
2. **ICDDR,B.** Anthrax outbreaks in Bangladesh: an update. *Health & Science Bulletin* 2010; **8**: December.
3. **Fasanella A, *et al.*** Detection of anthrax vaccine virulence factors by polymerase chain reaction. *Vaccine* 2001; **19**: 4214–4218.
4. **Van Ert MN, *et al.*** Global genetic population structure of *Bacillus anthracis*. *PLoS ONE* 2007; **2**: e461.
5. **Garofolo G, *et al.*** SNR analysis: molecular investigation of an anthrax epidemic. *BMC Veterinary Research* 2010; **28**: 11.
6. **Smith KL, *et al.*** *Bacillus anthracis* diversity in Kruger National Park. *Jounal of Clinical Microbiology* 2000; **38**: 3780–3784.
7. **Fasanella A, *et al.*** Severe anthrax outbreaks in Italy in 2004: considerations on factors involved in the spread of infection. *New Microbiologica* 2010; **33**: 383–86.
8. **Bangladesh Bureau of Statistics.** District profile, 2007 (http://www.bbs.gov.bd/RptZillaProfile.aspx). Accessed 25 April 2011.
9. **Hugh-Jones M, Blackburn JK.** The ecology of *Bacillus anthracis*. *Molecular Aspects of Medicine* 2009; **30**: 356–367.