ANTHRAX IN TEXTILE WORKERS

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Abstract

Anthrax continues to pose a threat to workers handling certain raw animal fibres. The disease is endemic in many countries, including some of those from which goat hair and wool are imported. Dust from anthrax contaminated fibres can cause potentially fatal pulmonary or skin forms of the disease. However, while anthrax is still being detected in imported fibres, the incidence of the human disease in the UK has fallen sharply; for reasons that are not entirely clear.

The paper describes research in the Health and Safety Laboratory into disinfection and the development of molecular based (PCR) testing methods for *Bacillus anthracis*. The paper also discusses the deregulation pressure to remove UK import restrictions and explores what other legislative controls might be appropriate.

Introduction

Anthrax is a potentially life-threatening zoonotic disease (disease spread from animals to man) acquired from infected animals and their products. It is endemic in some areas of the world, maintained in the animal host through gastrointestinal infection. Infection follows ingestion of spores as the animal grazes on infected land and during the course of the infection the spores are defaecated onto the grazing land, thus perpetuating the cycle. Hair, hides and wool can be contaminated by the spores, either in dust from the soil or in faecal material.

The human disease of anthrax is in three forms:

1. cutaneous or skin anthrax, resulting from skin contact with infected animals or products;

2. pulmonary or inhalation anthrax, causing virulent haemorrhagic bronchopneumonia following inhalation of spore-containing material; and

3. gastro-intestinal anthrax caused by ingestion of infected meat (mainly in Africa and Asia).

Risk to Textile Workers

It was in the textile industry that the first case of occupationally acquired anthrax in the UK was recorded in 1847 and the subsequent increase in incidence of the disease was attributed to the importation of alpaca and mohair (goat hair). Most cases arising from contact with these finer wools were seen in the mills in the town of Bradford and hence the disease became known as 'Bradford disease', or 'wool-sorters' disease' as it was called by the workers.

Historically, wool-sorters' disease was most frequently encountered in workers whose job was to open fleece bales and sort them into different qualities or to feed the animal hair into process machinery. However, the disease has also been reported in the later stages of processing. So, while the risk of contracting anthrax may be greatest in the early stages of animal hair treatment, cases have been encountered in e.g., carding, combing, drawing and spinning the hair.

In recent years all textile cases have involved cutaneous anthrax.

Prevalence of Anthrax

Data for animal infection world-wide has shown that about a quarter of the 174 countries monitored report disease in sheep and goats. These included Africa (Chad and Guinea Bissau) and Asia (Bangladesh, Bhutan, Iran, Pakistan and Turkey) (Fujikura, 1990).

Although human occurrence data available world-wide is patchy, significant outbreaks are reported in several countries. Table 1 summarises major outbreaks reported during the 1970s and 1980s. Sporadic outbreaks also occurred in 37 other countries. The vast majority of cases in these countries are gastro-intestinal, which contrasts with the situation in industrialised countries in temperate zones. For example, in USA, over 95% of all cases reported up to 1986 were cutaneous and 5% inhalation, with no adequately documented cases of gastro-intestinal disease. This equates to seventeen reported cases of inhalation anthrax since 1900 in USA, eleven since 1955. Since 1955 in USA, approximately 80% of cases have been industry related and 20% related to agriculture (Brachman, 1986).

A recent world-wide picture of livestock anthrax is described by Hugh-Jones (1996). In Europe, the major afflicted regions are Turkey, Greece, Albania, southern Italy, Romania and central Spain, and in Asia the area from Turkey to Pakistan remains severely affected. In 1994, Syria reported an epidemic status for sheep and goats. It was the author's (Hugh-Jones, 1996) opinion (notwithstanding some inconsistencies in reporting), that a belt of high risk areas for animal anthrax extends from the Mediterranean to the Pacific Ocean.

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Situation in the UK

In the first 40 years of this century 925 cases of anthrax arising from contact with wool and hair were reported in the UK; of these 146 were fatal (Chief Inspector of Factories - annual reports).

Human cases of anthrax are now exceedingly rare. Since 1981 there have been 17 human cases notified, from all sources. One confirmed case reported in 1991 was of cutaneous anthrax in a woollen mill worker in Scotland and one case was reported in 1992, but was unconfirmed. Only one case has been reported since then, in 1995 when a case of cutaneous anthrax was confirmed in a male casual labourer in London. Being a peripatetic worker, the source of the infection was not established, but he was in contact with imported leather goods and textiles (Breathnach *et al*, 1996). There have been no human deaths from anthrax reported in the UK since 1974. In the decades from 1960 total UK human anthrax cases have been 90, 36, 7, 6 (in the years 1990-1996).

Thus it could be argued that human infection in the UK has been eliminated. Nevertheless, it is a life threatening disease and as such requires continued control to ensure that no further infections occur.

What must also be considered is the issue of public perception. Anthrax is a publicly well known (but not well understood) and feared disease. Therefore any animal or human infection receives a high media profile which may be disproportionate to its significance, but which has a commercial influence on those industries such as textiles and leather where the potential for contamination still exists.

Legislative Controls

In the UK, goat hair is imported primarily from Asia and the Middle East (cashmere) and from South Africa and USA (mohair). Early in the 20th century, to curb the large number of deaths from anthrax in the textile industry, restrictions were placed on the importation of goat hair based on the county of origin. Little evidence existed at the time to correlate the risk of inhalation anthrax to the country of origin or the quantity of material being handled. The restrictions were specified originally by the Anthrax Prevention Act 1919 and associated orders, the latest of which is the Anthrax Prevention Order (APO) 1971.

Occupational health and safety law in the UK is the responsibility of the Health and Safety Executive (HSE). The APO is currently administered by HSE through its Textiles National Interest Group in Leeds.

Under the APO, animal hair imported from some countries or areas of countries designated as 'high risk' for contamination by anthrax spores (e.g., cashmere from Afghanistan), must undergo obligatory disinfection and must have a certificate of disinfection. For other 'questionable' or 'medium risk' countries (e.g., for common goat hair from China), animal hair is considered on a case by case basis, as it is brought into the country. Based on judgement which takes into account the nature and origin of the animal hair, a particular batch may be sent for disinfection immediately, or it may be held in quarantine until samples are taken and the results of analysis for anthrax contamination are known, or it may be passed for import without further treatment. Imports from 'no risk' countries need not be decontaminated, nor is there an obligation for samples to be taken, if the imported material is supported by a valid certificate of origin.

Efforts have been made to compare the UK situation with legislative measures taken in other countries. Some European countries also have specific controls, but it is not known how rigorously these are enforced or how effective they are in controlling risks in textile factories. European Community (EC) Directives on biological agents and animal products are also relevant but have yet to be shown to be effective in controlling the hazard of anthrax infection from imported materials. Further afield it is known that China has laws regulating the import of cashmere from Iran and Afghanistan, and requiring disinfection by fumigation with ethylene oxide and methyl bromide.

Fumigation is not recommended by HSE as a reliable method of disinfection due to the difficulty in ensuring that the fumigant can penetrate clumps of hair from the opened bale, or reach spores trapped in raw greasy material, dried clots of blood or faeces.

The UK recommended disinfection method is immersion in 0.9% formaldehyde solution for 1 minute, a variation of a method which was originally validated in 1918. As no recent scientific work had been done the Health and Safety Laboratory (an agency of the HSE) was asked to carry out research.

Laboratory and On-site Examination of the Effectiveness of Formaldehyde and Heat Treatment for Decontamination of Cashmere

Background

The recommended method to decontaminate cashmere goat hair potentially contaminated by anthrax spores involves immersion in a solution of formaldehyde during the scouring (cleaning) process followed by heating to remove excess moisture from the hair. The combination of chemical and physical methods was considered to be necessary to inactivate *Bacillus anthracis* spores efficiently, but firm scientific evidence to confirm this was limited. A series of laboratory tests was therefore undertaken to examine the efficiency of heat and formaldehyde treatment.

An examination was also made of the method as used in a factory that scours and decontaminates cashmere goat hair. This was to determine whether other variables such as heavy

organic loading or inconsistent heat and chemical penetration, not addressed in the *in vitro* experiments, could affect performance. To test the factory procedure, a spore tracer was put through the scouring and decontamination process.

Materials and Methods

A laboratory procedure was designed and validated to assess the performance of the recommended decontamination treatment on the kill rate of *Bacillus* bacterial spores. For safety reasons, most of the work was done using *B. cereus* as a surrogate for *B. anthracis*. This is consistent with other similar work (Jones and Turnbull, 1996) and the similarity in disinfectant susceptibility between *B. cereus* and *B. anthracis* was verified in initial experimentation.

B. cereus was grown in conditions to encourage sporulation (Reif *et al*, 1994), which yielded a suspension containing >90% spores. Volumes of the desired concentration of formaldehyde were warmed to 43ºC (the scouring temperature in the recommended method) and spore suspension was added at time 0 to give a concentration of 10^6 spores /ml. Portions were removed after one minute, immobilised onto glass fibre filters and exposed to 90^0C heat for different durations. They were then resuspended in a previously validated neutralising solution which negated the residual effect of the disinfectant, and 0.1 ml volumes of serial dilutions of the suspensions were spread onto the surface of Brain Heart Infusion Agar (BHIA), without blood added. BHIA was found to recover stressed spores better than other less nutritionally rich media (Bowry and Crook, unpublished data). The plates were incubated aerobically at 35^0C and colonies counted daily until no more emerged.

Testing the Factory Disinfection Process

Spores of *Bacillus subtilis var. globigii* were used as a tracer to test the performance of the factory decontamination process. This species is not phylogenetically as close to *B. anthracis* as is *B. cereus*, so there could be some small differences in susceptibilities, but it is frequently used as a tracer because it forms colonies with a distinctive red colour which differentiates it from most other bacterial colonies. Spore suspensions were prepared as described above and mixed with sterilised unscoured goat hair which was then sewn into 30 cm square muslin bags. Some prepared bags were retained and not exposed to the decontamination procedure to act as positive controls. The remaining bags were put through the decontamination treatment by placing them with other cashmere onto the feed conveyor. They went through a series of scouring bowls, the last of which contained formaldehyde solution, then through the heater/dryer and were retrieved. Each was placed individually in a sealed plastic bag, stored overnight at 4^0C then tested next day. From each sample, 5 g was taken and washed in mineral salts solution to resuspend the spores. The suspension was diluted in a tenfold series and 0.1 ml portions spread onto the surface of BHIA plates. These were incubated as above and the number of colony forming units (CFU) counted, from which the total yield per gram of original material was calculated.

Results and Discussion

Previous work by the Health and Safety Laboratory had shown that formaldehyde alone and high temperature alone were limited in their effectiveness at killing the *Bacillus* spores (Crook *et al*, 1996), but the hypothesis that a combination of heat and formaldehyde would be effective was proved, for formaldehyde down to 0.3% concentration followed by 15 to 20 min. heating at 90^0C (Table 3). The heat treatment is consistent with that used in factory conditions for decontamination of goat hair. Tests done using spore tracers in an end-use test at a factory showed that a substantial reduction in spore numbers could be achieved. Surviving spores were found to be 0.5% or less compared to controls (Table 4).

Development of a Molecular Based Detection System for Detecting *Bacillus Anthracis* in Cashmere Goat Hair

Background

The use of molecular based (Polymerase chain reaction - PCR) techniques, in which target DNA sequences are amplified prior to detection, potentially increases the speed, sensitivity and specificity of microbial detection in environmental samples. If coupled with an efficient method for extracting DNA from the physically tough bacterial spores, this offers an improved technique to monitor cashmere goat hair samples for the presence of *B. anthracis* spores and to improve worker health protection.

In multiplex PCR more than one sequence of DNA is amplified simultaneously using extra pairs of primers. In some cases, these sequences are different sections of the same gene (Dieffenbach & Dveksler 1995). Nested PCR is a technique where, to improve the sensitivity and specificity, a second set of primers are designed to amplify a region within the product generated by the first PCR process. After the first round of PCR cycles, 1µl of the product is added to a new tube containing the second set of primers and another PCR reaction is begun. This is the final product which is detected.

The aim was to develop a multiplex nested PCR to amplify sequences from the chromosome and pX02 virulence plasmid of *B. anthracis* which would simultaneously determine the presence of spores and their virulence with high sensitivity.

Materials and Methods

Bacillus cereus was used as a model for *B.anthracis* in developing a DNase treatment and bead beating protocol to release spore DNA. The spore suspension was treated with DNase (Boehringer Mannheim) to remove extracellular DNA, then the DNase was inactivated so as not to affect DNA subsequently released from spores. The samples then were processed in a FastPrep FP120 bead beater (Bio101,

CA, USA) which mechanically disrupts the bacterial spore walls, followed by a DNA extraction protocol done in the reaction tube which eliminates potential losses of DNA during transfer.

B. anthracis DNA was kindly supplied by P.C.B.Turnbull, CAMR, Porton Down, UK. The polymerase chain reaction (PCR) was performed using dNTPs and PCR buffer (Promega, UK) with previously optimised primer and magnesium chloride concentrations. This solution was overlaid with mineral oil (Sigma) and the DNA sample was added. After heating at 95^0C for 5 minutes in a thermocycler (Hybaid Omnigene), *Taq* DNA polymerase (Promega) was added to each PCR tube and cycling continued for 30 cycles of 1 minute each at 95^0C, 57^0C (or 52^0C for nested PCR) and 72^0C. A final cycle included an increased extension time of 7 minutes.

A pair of primers (926F and 1492R) which universally amplify a sequence from bacterial 16S rDNA were used in PCR as a positive control (Relman 1993). As the primers AR1 and AR2 employed by Patra *et al* (1996) are highly specific for *B.anthracis*, these formed one half of our multiplex with an internal pair designed for nested reaction. The other sequence to be amplified was taken from a *trans*-activator gene of encapsulation (acpaA) on plasmid pX02 from which sequences for initial and nested reactions were taken (Vietri e*t al* 1995).

All PCR products were visualised on a 1.5% agarose gel, run at 100V for 1 hour in TAE buffer (Sigma), followed by ethidium bromide staining for 30mins.

Results and Discussion

The bead beating preparation step was found to reduce the viability of *B. cereus* spores by more than 90%, demonstrating its efficiency at releasing spore DNA for subsequent detection.

By using a nested multiplex PCR two sequences from *B. anthracis* could be amplified: 150bp sequence from the chromosome (from primers AR1 & AR2), and 800bp section of the virulence plasmid pX02 (using primers acpA3 & acpA4). The 600bp positive control product was also amplified (with primers 926F & 1492R). If amplification of the pX02 sequence occurs it can be concluded that virulent organisms are present in the sample. The assay developed was specific for *B.anthracis* and could differentiate it from other *Bacillus* species.

Using 1:10 serial dilutions of DNA from 10ng to 1fg, the sensitivity of the nested PCR reaction was tested. The two sets of AR chromosomal primers were able to detect 1fg of starting DNA. This is equivalent to the detection of one *B. anthracis* genome per PCR.

The aim was to develop a rapid, specific and sensitive protocol for the detection of *Bacillus anthracis* spores in commercial samples of wool and cashmere. A nested, multiplex PCR method was produced which could detect sequences from both the chromosome and pX02 virulence plasmid of *B. anthracis*. The whole detection protocol potentially is more rapid than conventional current microbiological tests by culturing; sample preparation, DNase treatment, bead beating and DNA extraction would take approximately half a day, each half of the nested PCR takes 3 hours to complete, then agarose gel electrophoresis and ethidium bromide staining can be finished within 1 hour.

Legislative Change

The legislative controls in the UK for most occupational health hazards have in recent years been brought together into a single code of regulations- the Control of Substances Hazardous to Health Regulations (COSSH). These are "goal setting" regulations which require, amongst other things, the controls at the workplace to be based on risk assessments carried out by the employer.

The Regulations on anthrax in the textile industry do not fit in with this approach since they are highly prescriptive and are based on a system of authorisations which is out of proportion to the risk as judged by present day experience. They are also defective in a number of ways as an effective regulatory control. Yet it has proved impossible so far to convince the industry and the trades unions that the Regulations should be revoked in favour of a reliance on the more general COSHH Regulations.

Advocates of the retention of the Regulations argue that the present low incidence of the disease could be an indication of their effectiveness as a control regime. An alternative explanation is that it is at least as much due to improvements in public health and hygiene, and to improved conditions in the mills.

It is hoped that the recent work on disinfection processes and spore detection will give both sides of the industry confidence that a move to a less prescriptive regime will not lead to reduced protection for the workers in the industry.

Conclusion

Anthrax is a potentially fatal disease which is endemic in certain parts of the world. Much of the raw material for luxury textile products is sourced from such regions and may therefore be contaminated with anthrax spores, presenting a risk of infection to workers in the textile industry.

Techniques for the disinfection of textile fibres have recently been re-examined and validated in the UK in both *in vitro* and practical tests. A new molecular-based method for detecting the presence of *Bacillus anthracis* in cashmere has also been developed and provides a faster method of testing than conventional microbiological techniques.

These techniques, allied to a sound sampling protocol, provide for the reliable assessment of risk of anthrax from textile fibres and for the disinfection of infected material. In the UK this should enable greater confidence to be placed in a legislative regime based on risk assessment and control.

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Table 1. Major outbreaks of human anthrax reported world-wide 1976 to 1986 (from Fujikura, 1990)

Year	Location	Reported Casualties
1976	Kenya	327
1976	Turkey	305
1977	Burkina Faso	497
1978	Mali	152
1978	Iran	730
1978	Vietnam	107
1979	Egypt	2043
1980	Mali	121
1980	Rwanda	300
1980	Iran	391
1981	Rwanda	249
1981	Senegal	150
1981	Sudan	1362
1981	Iran	182
1981	Thailand	116
1982	Thailand	251
1976-1980	Iraq	200 to 269 yearly
1976-1982	Spain	216 to 315 yearly

Table 2: Cases of anthrax notified under the factories acts, arising from contact with wool and hair: 1900 to 1996

	Total	Fatal
1900-1909	210	49
1910-1919	420	62
1920-1929	176	26
1930-1939	119	9
1940-1949	53	6
1950-1959	53	2
1960-1969	24	3
1970-1979	3	-
1980-1989*	1?	-
1990-1996*	1?	-

*Source: Chief Inspector of Factories (*and Public Health Laboratory Service)*

Table 3. Yield of surviving spores (CFU) after different exposure times to heat at 90^0C by Formaldehyde concentration

Conc'n	Time (minutes)							
		1	3	5	10	15	20	30
0%	8.1×10^5	3.7×10^5	3.3×10^5	3.7×10^5	2.1×10^5	2.4×10^5	1.2×10^5	
0.1%	4.6×10^5	4.2×10^5	3.5×10^5	3.2×10^5	3.4×10^5	3.1×10^5	9.8×10^2	3.5×10^2
0.3%	3.9×10^5	3.5×10^5	1.9×10^5	2.3×10^5	1.7×10^5	1.3×10^5	ND	ND
0.5%	4.1×10^5	4.1×10^5	4.0×10^5	3.0×10^5	7.0×10^4	ND	ND	ND
0.7%	4.8×10^5	3.7×10^5	3.3×10^5	3.2×10^5	1.4×10^4	ND	ND	ND
0.9%	5.3×10^5	5.1×10^5	5.2×10^5	3.2×10^5	4.0×10^4	ND	ND	ND

ND = Not detected

Table 4. Bacillus spore tracer recovery from cashmere samples treated by factory decontamination process

Tracer material	Treatment	CFU/GRAM tracer recovered	No. as % of control
Untreated cashmere seeded with *B.subtilis* spores: batch 1	Control	2.6×10^6	-
	Control#2	1.6×10^6	-
	Processed	3.7×10^3	0.10
	Processed	1.2×10^4	0.50
Untreated cashmere seeded with *B.subtilis* spores: batch 2	Control	3.4×10^6	-
	Processed	1.2×10^3	0.03
	Processed	1.5×10^3	0.04
	Processed	9.0×10^2	0.02