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1 Anthrax undervalued zoonosis

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12 13 **Abstract**

14
15 Anthrax is a non-contagious disease, known since ancient times but it became a matter of global public
16 interest after the bioterrorist attacks in the U.S.A. during the autumn of 2001. The concern of politicians and
17 civil authorities everywhere towards this emergency necessitated a significant research effort and the
18 prevention of new bioterrorist acts. But anthrax is primarily a disease that affects livestock and wildlife; its
19 distribution is worldwide; and it can represent a danger to humans but especially when it occurs in areas
20 considered to be free and in atypical seasons and climatic conditions. The atypicality of the phenomenon
21 may lead health workers to misdiagnosis and, consequently, an inappropriate management of affected
22 carcasses with a consequent and inevitable increase in the risk of human infection. This paper emphasizes the
23 importance of increasing attention to this zoonosis. The biggest risk is its underestimation.

24
25
26 **Key words:** *Bacillus anthracis*, animal, disease, zoonosis

30 **Introduction and historical perspective**

31 Anthrax is an infectious disease with clinical features overlapping the haemorrhagic fevers with hyperacute
32 or acute symptoms and usually with a fatal outcome. The responsible bacterium, *Bacillus anthracis*, was
33 identified only in the late 19th century thanks to studies by Robert Koch (1876).

34

35 Pasteur in 1881, with his famous experiment at Fort Pouilly, showed the world the opportunity to fight
36 anthrax with preventive vaccination, beginning the era of vaccines. However, *B. anthracis* with its high
37 pathogenicity has aroused also less noble interests. From 1932 to 1945 the bacteriologists of the Japanese
38 Army Unit 731 tested it as a biological weapon on Manchurian prisoners (Hudson *et al.*, 2008). Despite the
39 signing of a treaty banning bacteriological arms, an outbreak of human anthrax occurred in Sverdlovsk,
40 USSR, (now Ekaterinburg, Russia) in April 1979. Soviet officials attributed this to the consumption of
41 contaminated meat, but Western governments believed it resulted from inhalation of spores accidentally
42 released from a nearby military research facility. Tissue samples from 11 victims were obtained and
43 analyzed. Results demonstrated that the entire complement of *B. anthracis* toxin and capsular antigen genes
44 required for pathogenicity were present in the tissues from each of these victims. The DNA analysis
45 demonstrated that at least four different strains of *B. anthracis* were present in the tissue samples (Jackson *et*
46 *al.*, 1998). The world has learnt much about *B. anthracis* following the bioterrorist attacks in the autumn of
47 2001 in the United States, where five letters containing small quantities of anthrax spores contaminated more
48 than 30000 people, killed 5 people and infected 17 (Jernigan *et al.*, 2002). These events represent a turning
49 point in the history of highly pathogenic agents and their use as a means of aggression against civil
50 populations: *that which until then was considered only a remote probability had become a terrible reality*
51 *and a dangerous example that could be imitated by anyone.*

52

53 The concern of politicians and civil authorities towards this new emergency necessitated significant
54 preventative programs against new bioterrorist acts and in planning research projects whose aim was to
55 understand the detailed mechanisms of action of the pathogens, and, through therapeutic and preventive
56 means, to minimize the damage of a possible mass attack. We should not forget that anthrax is a disease
57 known since ancient times and it is still present sporadically and endemically in many areas of our planet.
58 The disease is characterized by outbreaks that usually involve a small number of animals, herbivores that are
59 usually kept on pasture, but it can sometimes turn into an epidemic with potentially very serious
60 consequences for humans.

61

62 **The infectious agent**

63 *B. anthracis*, belongs to the family of Bacillaceae. It is a Gram positive bacterium, a small rod in shape,
64 aerobic, immobile, capsulated, and forms spores. The bacterium is 1-1.5 μm wide by 5-6 μm in length. In
65 tissue or culture smears other pathological bacteria are found singly, as clusters or as short chains, with

66 rounded ends, while *B. anthracis* cells with square ends are arranged in long chains that gives them a
67 particular look similar to "bamboo canes" (*Fig.1*). Outside the body and at temperatures between 14°C and
68 42°C (optimum between 21°C and 37°C) *B. anthracis* will sporulate (*Fig.2*). The spores are oval, and are
69 released after lysis of the bacterium. Sporulation is completed within 48 hours, but it does not happen in the
70 presence of high concentrations of CO₂, a condition that occurs in infected putrefacting carcasses.

71

72 *Culture media features*

73 *B. anthracis* grows well in ordinary medium under aerobic or microaerophilic conditions, at temperature
74 between 12°C and 44°C, but optimal growth occurs around 37°C and at a pH of 7.0 to 7.4.
75 On nutrient agar it forms white colonies 3-4 mm in diameter with a rough surface, called "glass beads"
76 (*Fig.3*), and with irregular margins that if observed at a small magnification have a medusa head appearance.
77 In tryptose broth there is a flocculation, which eventually settles on the bottom, leaving clear liquid above.
78 On blood agar it does not cause haemolysis, unlike other bacteria-like anthracoids, which haemolyze in a
79 more or less obvious way. It is normally sensitive to penicillin and various phages. When grown for 2 weeks
80 at a temperature of 42 °C it will lose its virulence in a specific manner (a.k.a. *Pasteur vaccine strains*). This
81 weakening is due to the loss of the pXO1 plasmid on which the toxic factors are encoded. The colonies of
82 attenuated strains are smaller, smooth, and convex.

83

84 *Resistance*

85 Vegetative forms are not very robust and they are inactivated within 30 minutes at 60°C -65°C (Turnbull,
86 1998). The great strength of *B. anthracis* is linked to sporogenesis which is nothing else but a form of
87 bacterial resistance to living under unfavorable conditions. The spores are destroyed only after ten minutes at
88 boiling temperature but can withstand 98°C for 30 minutes (personal observation); they are destroyed in 20
89 minutes in an autoclave set at 121°C. Spores survive well in dried and salted hides, and at low temperatures.
90 The normal fixation techniques do not kill the spores, which can successfully germinate even after many
91 years, so it is necessary to flame slides several times before assuming the spores are dead. The spores are
92 sensitive to 2%-3% formaldehyde solutions at 40°C for 20 minutes, or 0.25% at 60°C for 6 hours, or at 4%
93 after a contact of at least 2 hours. The spores are destroyed by 5% phenol and mercury chloride, and 1%
94 solutions of caustic soda and potash. Spores in soil can persist for many years, but ultraviolet rays will
95 inactivate them in a few hours.

96

97

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102 **The major virulence factors of *Bacillus anthracis***

103 The pathogenic action of *B. anthracis* is closely linked to toxic factors, resulting from two plasmids:
104 • **pXO1**, 182 Kb, on which the genes encoding the oedema factor (EF), the lethal factor (LF) and the
105 protective antigen (PA) are located;

106 • **pXO2**, 96 Kb, on which the genes encoding the biosynthesis of the capsule are located (Uchida I., *et*
107 *al.*1985)

108 The complete chromosomal and plasmid DNA sequences from multiple strains are available from public
109 databases. *B. anthracis* expresses its pathogenic action mainly through the capsule and the production of a
110 toxic complex consisting of three proteins known as protective antigen (PA), lethal factor (LF), oedema
111 factor (EF) .

112 The PA plays a fundamental role in the toxic action of *Bacillus anthracis*. In fact, this protein of about 83
113 kDa has no direct toxic action but acts as a "transporter" of the other two toxic proteins. PA binds to two cell
114 surface receptors, the tumour endothelium marker 8 (TEM8) and the capillary morphogenesis protein 2
115 (CMG2), both of which are expressed on many cell types, including cells of the immune system (Collier and
116 Young 2003; Scoobie and Young, 2005). The proteolytic release of the C-terminal domain (20 kDa) of PA
117 results in spontaneous oligomerization of truncated PA (PA₆₃) into heptamers (PA₆₃)₇, which bind EF and LF
118 (Collier and Young, 2003). The (PA)₇-EF and the (PA)₇-LF complexes enter rafts, inducing their endocytosis
119 inside acidic compartments (Abrami *et al.*, 2005). The low pH of the late endosomal lumen triggers a
120 conformational change of the complex, with insertion of part of PA into the membrane and translocation of
121 EF and LF into the cytosol (Abrami *et al.*, 2005). EF is a calmodulin-dependent adenylate cyclase (Leppla,
122 1982), which creates a gradient of c-AMP with a high concentration in the perinuclear area (Dal Molin *et al.*,
123 2006), while LF is a metalloprotease which cleaves most isoforms of MAPKKs (MEKs) throughout the
124 cytosol (Vitale *et al.*, 2000); this does not exclude that it may act on other cytosolic proteins, as suggested by
125 a recent report that LF acts on the inflammasome (Boyden and Dietrich, 2006). MEKs are part of a major
126 signalling pathway linking the activation of membrane receptors to transcription of several genes, including
127 those encoding pro-inflammatory cytokines and other proteins involved in the immune response. The capsule,
128 a linear polymer of γ -D-glutamic acid, is considered the other major virulence factor of *B. anthracis*. The
129 capsule contributes to pathogenicity by enabling the bacteria to resist phagocytosis by macrophages, and thus
130 evade the host immune defenses and promote septicemia. Additionally, the capsule is only very weakly
131 immunogenic. As well as demonstrating the relative contributions of the above factors to strain virulence
132 Coker *et al.* (2003) showed that the two plasmids were not singular but that their copy numbers, especially
133 for pXO2 the capsular plasmid, predicted virulence.

134

135

136

137 **Epidemiology**

138

139 Anthrax is a disease known to man since ancient times and the first reports appear in the Bible (Exodus,
140 Chapters 7 to 9) as the characteristics of the fifth and sixth plagues, which struck Egypt around 1491 BC, are
141 identifiable with anthrax. Even writers in ancient times (Homer: Iliad I. 49-50, Trojan war, 1228 BC; Livy:
142 Rome, 461 BC; Plutarch: 753 BC; Aristotle: History of animals, 333 BC; Virgil: Georgics, 32 BC) describe a
143 livestock disease with the same characteristics as anthrax in their works (Blancou, 2003).

144

145 Between the late 19th and early 20th century anthrax was one of the infectious diseases with major mortality
146 among domestic and wild animals. Probably the most serious incident occurred in 1923 in South Africa
147 where in one year it killed between 30000 and 60000 animals (Sterne, 1967). Later with the appearance of
148 the Sterne vaccine, of antibiotics, and the beginning of cost effective programs for prevention and control,
149 the disease has lost its importance, taking on characteristics of sporadicity. Worldwide it is now an
150 uncommon disease in much of Western Europe, Northern America and Australia, with exceptions in
151 endemic foci in wild fauna in the African national parks (Hugh-Jones, 1999). In Canada it is enzootic in
152 specific locations in the North-West Territories (Slave River Flats) and Alberta (Wood Bison National Park)
153 (Nischi *et al.*, 2002), and has the potential if control is relaxed to cause epidemics in the Canadian Prairie
154 provinces, while in the U.S.A., the disease is a persistent threat in eastern North and South Dakota and
155 North-West Minnesota, is enzootic in South-West Texas (Hugh-Jones, 1999) and suddenly ‘appeared’ in
156 2008 in South-West Montana where it had been unappreciated for decades. In Australia, anthrax is sporadic,
157 although a sudden and severe epidemic occurred in Northern Victoria in 1997 (Turner *et al.*, 1999). In
158 Europe, the major enzootic areas are Greece, Spain, Turkey, Albania, France, and Southern Italy (Fouet *et*
159 *al.*, 2002; Fasanella *et al.*, 2005), but it is essentially absent from Northern Europe.

160

161 While the incidence is generally decreasing worldwide it persists in certain countries; for example it is
162 hyper-enzootic in Haiti and still enzootic in Bolivia, Mexico, and Peru. This follows from ineffective control
163 programs. In contrast, vaccination programs in Belize, Nicaragua and Chile have resulted in good control. It
164 is still absent from the Guianas. In Russia and in countries of the former Soviet Union, lack of effective
165 control programs is evidenced by the high percentage of human cases, reflecting the inadequacies of both the
166 public health systems and the veterinary services (Hugh-Jones, 1999). In Asia, anthrax is widespread in the
167 Philippines, South Korea, Eastern India, and in mountainous zones of Western China and Mongolia; porcine
168 anthrax is frequently reported in the highlands of Papua New Guinea. Africa remains severely afflicted, with
169 major epidemic areas in wildlife areas such as Queen Elizabeth National Park (Uganda), Mago National Park
170 Omo (Ethiopia), Selous National Reserve (Tanzania), Luangwa Valley (Zambia), Etosha National Park
171 (Namibia), Kgalagadi Transfrontier Park (Botswana and South Africa), and Vaalbos and Kruger National
172 Parks (South Africa). (Ebedes, 1976; Turnbull *et al.*, 1991b; Hugh-Jones and de Vos, 2002). An anthrax-like
173 disease has been found in wild non-human primates living in tropical rainforest, a habitat not previously

174 known to harbour *B. anthracis* (Leendertz *et al.*, 2004). It was characterized by an unusually high number of
175 sudden deaths observed over nine months in three communities of wild chimpanzees (*Pan troglodytes resus*)
176 in the Tai National Park, Ivory Coast. However, *Bacillus* strains associated with this outbreak were toxigenic
177 *B. cereus* and not typical *B. anthracis*.

178

179

180 **Receptive animals**

181

182 The sensitivity to anthrax varies considerably between the different animal species. In nature herbivores are
183 particularly susceptible and omnivores and carnivores are moderately resistant but still succumb. The species
184 most susceptible to infection, in descending order, are: cattle, sheep, horses, pigs, goats, and camels. Among
185 herbivores, in addition to domestic species, including donkeys and mules, wild species are also susceptible
186 and in particular zebras and the wild ruminants such as deer (Hugh-Jones and de Vos, 2002; Fasanella *et al.*,
187 2007) buffalo, gnu, gazelles, kudu, antelopes, and reindeer. Outbreaks have been reported in elephants, in
188 some wild omnivores such as warthogs and bush-pigs, and among carnivores, as well as in the domestic dog
189 and farmed mink. The disease has been reported in the national parks in Africa in wild dogs (lycaons,
190 jackals), hyenas and the big felines (lions, cheetahs, leopards). Pigs and scavengers are more resistant to the
191 disease than herbivores and they must ingest a large volume of bacilli to contract the infection. The greater
192 susceptibility of bovines is related to their ease of getting infected and to the grazing habits of this animal. In
193 the absence of upper incisors, they rip up the grass with their tongues, and this action facilitates the ingestion
194 of soil, which is the main vehicle of bacillus spores.

195

196

197 **Transmission**

198

199 Anthrax is not contagious from sick to healthy animals, but by the ingestion of spores dispersed into the
200 environment. It's widely believed that the vegetative forms of *B. anthracis* sporulate when exposed to
201 oxygen. Under these assumptions it is assumed that in an intact carcass putrefactive processes should destroy
202 almost all bacteria in a period of time ranging from 48 to 72 hours (Stein, 1947a). But rarely in nature are
203 carcasses of dead animals left undisturbed (*Fig. 4*) by scavengers. Since the carnivores are less susceptible to
204 the disease compared to herbivores, they can ingest larger quantities of infected meat and then spread spores
205 in their feces (Pienaar, 1967; Turnbull *et al.*, 1989). During an epidemic in Basilicata in the summer of 2004,
206 anthrax spores of the same genotype responsible of the epidemic were isolated from wild boar feces (not
207 published). In rural areas scavenger birds such as ravens (*Corvus corax*) and vultures (various spp), can
208 contaminate pastures or small bodies of water far from the original outbreak (Pienaar, 1967). Another form
209 of pasture contamination occurs when necropsies are performed on-site by uninformed veterinarians or by
210 farmers salvaging the hide by skinning a carcass. Even if the carcasses are deeply buried, the spores can be

211 conveyed to the surface by the ground water or by land movements (Turner, 1999). In the past, washing
212 waters from tanneries and wool mills were run off in water courses or used as fertilizer. Today outbreaks are
213 associated with the use of soil contaminated forages produced on “champs maudit” contaminated land, and
214 to products of animal origin such as meat and bone meals derived from the inadequate processing of infected
215 carcasses. These products are very dangerous because they can cause unexpected outbreaks in conditions and
216 situations epidemiologically very different from the classic episodes of telluric origin. The danger is that they
217 can occur silently anywhere, even where anthrax is unknown, and at any time of the year.

218

219 Laboratory studies have shown, using mouse and guinea pig models, that stable flies *Stomoxys calcitrans* and
220 *Aedes aegypti* and *Aedes taeniorhynchus* mosquitoes are able to transmit the infection. The percentage of
221 transmission is very low (about 17% in the flies and 12% in the mosquitoes) but it is suspected that when the
222 insect population density is high, they could be an important vehicle in the spread of the disease (Turell and
223 Knudson, 1987) . The role of tabanid *Haematobia irritans* in the spread of the disease was confirmed in two
224 old scientific papers (Mitzmain, 1914; Morris, 1918). Lately Tabanid flies are considered to be of greater
225 importance. (Kranevelde and Djaenodin , 1940; Rao and Mohiyudeen, 1958; Davies, 1983; Blackburn, 2006;
226 Hugh-Jones and Blackburn, 2009)

227

228

229 **Pathogenesis**

230

231 The most common way of penetration by spores is via the digestive tract after the ingestion of spore
232 contaminated feed, forages, and water. The ports of entry are micro-wounds that can be found in the mucous
233 membranes of the mouth, pharynx and along the entire gastrointestinal tract. The infection can also occur
234 through skin abrasions or skin lesions that may be caused by haematophagous insects (e.g. biting flies) acting
235 as passive carriers or biological vectors. Although less frequent, spread is possible through the inhalation of
236 dust containing spores. The severity of the disease depends on the sensitivity of the host, on the infectious
237 dose, and on the route of penetration. Regardless of the route of penetration, it is considered that the spores
238 of *B. anthracis* are carried by macrophages from the initial site of entry to the draining lymph nodes. The
239 spores germinate, giving rise to vegetative forms that are capable of producing the main virulence factors:
240 toxins and capsule.

241 Whatever the route of infection, it is believed that *B. anthracis* spores are transported by macrophages from
242 the original site of introduction to draining lymph nodes and then enter the blood stream where they continue
243 to multiply rapidly . The pathogenicity of *B. anthracis* depends on the quality of the capsular coat and the
244 amount of toxins produced (Coker *et al.*, 2003; Shoop *et al.*, 2005) as well as on the sensitivity of the host
245 species (Smith, 1973). In "Fisher 344" rats, the injection of the toxin causes death in about 30 minutes and a
246 severe pulmonary oedema can be seen. Rabbits experimentally infected with *B. anthracis* show respiratory

247 symptomatology due to the intense action of the oedematous toxin on the lung. The leakage of blood from
248 the nose is always just before or just after the death of the animal (personal observations).

249

250

251 **Symptomatology.**

252

253 *Cattle and sheep*

254 Bovine anthrax may be hyperacute or acute. In the hyperacute form animals are frequently found dead
255 without the owner noting any obvious premonitory signs. This usually occurs at the beginning of an
256 outbreak. In animals where clinical signs are observed, this will include fever, wheezing, congestion of
257 mucous, muscle tremors, and convulsions. Death is sudden from shock and occurs within a few hours and is
258 occasionally accompanied by the extravasation of blood from the nostrils, mouth, anus, and vulva. In the
259 acute form the disease is characterised by septicemia, high fever (41°C-42°C), and tachypnoea, congested
260 and haemorrhagic mucous membranes. Initially the animal may be excited, but this is followed by major
261 depression. There is a loss of appetite and ruminal stasis. Pregnant cows may abort and those lactating may
262 demonstrate a drop in milk production. Cutaneous oedema sometimes appears in the neck and ventral parts
263 of the chest and abdomen. The animals die in 48-72 hours from shock.

264

265 In the sheep the fulminant form is most common: the animals can die in a few minutes with convulsions.
266 Less acute affected animals stop eating, have high fevers, dyspnoea, muscle tremors and gnashing of teeth.
267 Sometimes they will emit a reddish foam from the mouth shortly before dying.

268

269 *Equines*

270 The clinical manifestations and the course of the disease are almost always of an acute form with death
271 occurring in 2-3 days. The disease develops with colic syndrome and septicemia associated with muscle
272 tremors, sensory depression, a very high fever, cyanosis, tachypnea, and tachycardia.

273

274 *Pigs*

275 This species is more resistant and the disease is usually subclinical (Smith, 1973). It manifests as a localized
276 swelling in the pharynx –the so-called "anthrax angina"– or in the intestine. If the location is in the throat
277 there will be oedematous hot and painful swelling of the parotid region which may extend to the neck and
278 chest, depression and fever, with anorexia or dysphagia, and cyanosis of the mucous membranes. There may
279 be a profuse diarrhoea after an intestinal infection. When the lesions are severe, death occurs within 3-7
280 days. With only retropharyngeal or mesenteric lymph node inflammation the subject recovers. It seems that
281 the nature of the contaminated feed can play an important role since a fibrous abrasive feed can kill while the
282 same spore dose in a soft feed will pass through the pig without apparent harm. (Ferguson, 1981)

283 *Carnivores*

284 These animals are fairly resistant but if affected they show signs of acute gastroenteritis and oro-pharyngitis
285 which is due to ingestion of large volumes of infected meat. Usually recovery occurs spontaneously.

286

287

288 **Pathology**

289

290 Septicemic anthrax includes oedema, haemorrhage and necrosis (Gleiser, 1967). The lesions vary depending
291 on the route of infection, the sensitivity of the host, and the virulence of the bacteria. Thus some animals
292 show only signs of septicaemia, while in others just necrotic lesions occur (de Vos, 1994).

293

294 *Cattle and sheep*

295 In ruminants the disease is characterised by splenomegaly, bleeding and diffuse oedema predominantly in the
296 connective tissues (Marcato, 1981). The carcass rapidly decomposes and swells (de Vos, 1994); *rigor mortis*
297 is incomplete; and blood is dark red, is uncoagulable, and sometimes extravasates via natural openings
298 (nostrils, mouth, anus, vulva). The blood clots are gelatinous because the normal blood coagulation processes
299 are altered. This is accompanied by cyanosis and apparent mucosal bleeding, a gelatinous infiltration of the
300 subcutaneous connective tissue, and congestion of the serosa, often with hemorrhagic petechiae (Contini,
301 1995), which collect a blood coloured liquid, particularly in the peritoneum, pleura and pericardium.
302 Haemorrhages can be found throughout the internal organs. Sometimes small quantities of serum sweat from
303 tissues of the neck and inguinal regions (Marcato, 1981). There may be blood mixed with urine in the
304 bladder (Contini, 1995). The organ with the greatest changes is the spleen (de Vos, 1994), which has
305 congestive-hemorrhagic tumefactions in the red pulp as a result of septicaemia. There is a significant
306 increase in the volume of this organ and the capsule is tense; on dissection the pulp is red and black and the
307 white pulp hard to see (Marcato, 1981). But splenomegaly is inconstant. The lesions may also affect the
308 intestine; the internal mucosa is hyperaemic and full of punctiform haemorrhages. There are round
309 tumefactions in the lymphoid tissue of the Peyer's patches that are hemorrhagic-necrotic and ulcerative. The
310 lesions can extend to the mesentery. Hemorrhage and oedema may be found in relation to the pharynx,
311 larynx and lungs (Contini, 1995). Sometimes there are cases of cutaneous oedema because of a local
312 infection (Marcato, 1981). Sheep are less resistant than cattle and therefore in these animals the disease
313 develops faster.

314

315 *Equines*

316 In horses anthrax involves oedematous subcutaneous swelling of the neck, shoulders, chest, abdomen, and
317 perineum (Sterne, 1959). The cutaneous oedema suggests a cutaneous reaction to bites from contaminated
318 horseflies. When there is an infection of the pharynx or intestine from contaminated feed or forage there is
319 often a diffuse hemorrhagic ulcerative enteritis. The regional lymph nodes are red and swollen with

320 yellowish areas of necrosis. Splenic lesions will be absent if the animal dies as a result of local reaction,
321 without septicemia.

322

323 *Pigs*

324 In pigs the primary lesions are located in the pharynx and intestine as a result of the ingestion of infected
325 meat leading to the formation of the so-called "angina anthrax". There is a hemorrhagic oedematous swelling
326 of the mucosa and sub-mucosa of the pharynx, glottis, peripharyngeal tissues, and of the subcutaneous
327 connective tissue of the throat and neck (Henning, 1956). This is characterized by diphtheric membranes on
328 the surface and deep, hemorrhagic, necrotic, grey-yellowish grey-brownish processes (Marcato, 1981). The
329 regional lymph nodes – sublingual, retropharyngeal, sub-parotid – increase to several times their normal size.
330 They are coloured dark red because of the adenopathy from the oedema, the iperemia, the haemorrhage and
331 secondary necrosis (Ferguson, 1981). Anthracis pustules may form in the intestines and can be localised or
332 diffuse, with hemorrhagic areas of inflammation affecting the wall of the intestine and corresponding
333 mesentery. Only the mesenteric lymph nodes may be affected (Henning, 1956).

334

335

336 **Diagnosis**

337

338 Suspicion of anthrax arises from the observation of clinical symptoms, the anatomic-pathological findings
339 and epidemiological data. The ecology of the bacterium limits the distribution of the disease that is almost
340 always confined to the well defined territories, in which one can observe, with systematic cyclicality, sporadic
341 outbreaks usually involving a few animals (one or two on average), but the frequency of which tends to
342 increase during dry summers that follow very wet springs (Hugh-Jones, 1999). Less frequent and certainly
343 more dangerous are introductive events that affect animals living in fixed stables which contract anthrax by
344 eating contaminated food (usually forages) coming from high risk areas. This can and does happen in areas
345 normally deemed free of anthrax and commonly in winter when livestock needs extra feed which will have
346 to be purchased and may be contaminated. Thus, despite a careful epidemiological analysis, this can lead
347 health professionals to misdiagnose suspect cases and consequently, the subsequent inappropriate
348 management of infected carcasses that leads to an inevitable increase in the risk of infection in humans and
349 other livestock. (Kreidl *et al.*, 2006).

350

351 *Differential diagnosis*

352 In cattle anthrax should be differentiated from the following diseases:

- 353 ▪ lightning strike and accidental electrocutions
- 354 ▪ pasteurellosis
- 355 ▪ piroplasmosis
- 356 ▪ blackleg, malignant oedema and other clostridial diseases

357 ▪ food intoxications

358 However one should consider any disease causing sudden death or hemorrhagic septicaemia. In horses we
359 should consider colic syndromes, because of their symptomatology and infectious anaemia and dourine,
360 because of the oedemas. However, in infectious anaemia, sublingual haemorrhages can be found.

361

362 *Laboratory diagnosis*

363 When taking a sample from a dead animal suspected of anthrax one needs to take precautions to prevent
364 human infection, bacterial sporulation and a resulting environmental contamination. From live animals blood
365 can be collected from the main superficial veins; while from dead animals, it can be taken from the
366 peripheral veins, such as in the ear after removal of the auricle with a hot knife; in this way the wound is
367 cauterized and the spilling of blood and contamination of the floor by spores prevented. The blood can be
368 either on a cotton swab or in a vacutainer; the former is better. When using a cotton swab the blood should
369 be allowed to dry, killing contaminants and encouraging any *B. anthracis* to sporulate. Putrefaction quickly
370 destroys vegetative *B. anthracis*, which can therefore be difficult to isolate from the carcasses just 48 hours
371 after death, especially in hot weather (Stein, 1947b). Traditionally ears are collected as they are convenient
372 and far from the intestinal tract but a better sample are nasal turbinates which are well vasculated and
373 therefore should have plenty of spores but with minimal tissue that is only little affected by putrefaction. One
374 can also take serous liquids from oedematous areas and soft organs but these should be placed in leak-proof
375 containers. At low temperatures (5°C -10°C) it is possible to isolate the bacillus up to 4 weeks after death
376 (Whitford, 1979). However, in reality the sample should be taken as soon as possible since decomposition
377 leads to the rapid disintegration of the bacilli.

378

379 In the case of sheep or goats, one can send the entire carcass to the diagnostic laboratory in a leak proof
380 container with a label indicating the suspected disease. This precaution must be taken even with single
381 organs, such as the spleen or a collection of lymph nodes (in particular the prescapular node). When the
382 carcass is too dehydrated, which can present diagnostic problems, one can collect soil from the ground under
383 the animal that may have been contaminated by the leakage of blood and other body fluids from the natural
384 openings and seepage. It should be noted that the longer an animal has been dead the smaller is the
385 probability of getting a positive diagnosis, even with an experienced diagnostic laboratory.

386

387 *-Microscopic test*

388 A preliminary examination with an unstained fresh blood smear will highlight the presence of rod like forms
389 or typical "*bamboo canes*". The organisms are immobile and well capsulated. The slide may be fixed and
390 stained with Gram stain and *B. anthracis* is violet. Preferably one can use Giemsa stain which stains the
391 bacilli purple and the capsule a characteristic red mauve or with MacFadyean stain, which is blue methyl
392 polychromatic and stains the capsule pink. Löffler uses methylene blue to which K_2CO_3 to 1% has been
393 added (Turnbull, 1998). For *Bacillus anthracis* this leads to the metachromatic phenomenon with the bacterial

394 bodies stained blue while the capsule takes on a reddish colour. In the preparation of the slide one must take
395 care to pass the slide several times over the flame because the usual methods of fixing colours do not
396 inactivate the spores, which can represent a significant danger to the staff that will handle these microscopic
397 preparations. Anecdotally are stories of students getting cutaneous lesions from handling sharp-edged broken
398 blood smear slides that were decades old.

399

400 - *Cultural test*

401 *Bacillus anthracis* grows easily on normal agars, whether liquid or solid. Using a sterile loop the plates can
402 be sown with material from samples of blood, exudates, oedematous infiltrations, organs or parts of them
403 taken from infected or suspected animals. When one suspects the presence of spores in the material used in
404 the sample (wool, hair, leather, environmental samples) it is necessary to first incubate the material at 72°C
405 for 30 minutes to destroy contaminating bacteria, yeasts and moulds. It is always better to use a semi-
406 selective medium to isolate the bacterium. Moreover blood-containing media are preferable in comparison to
407 the often-used PLET or a Knisly agar, such as TSPB Agar, which is made highly selective against Gram-
408 negative bacteria by supplementation with trimethoprim (13.1 mg/L), sulfamethoxazole (20 mg/L) and
409 polymyxin B (30000 IU/L) (Tomaso *et al.*, 2006). The plates are then incubated at 37 ° C for 24 hours. If the
410 bacterium is present in the material used, white colonies will develop, 2-5 mm in diameter, of a pasty
411 consistency, and non-haemolytic. At a small magnification one can see long filaments folded several times
412 on their own that seem to have the appearance of the foliage of a jellyfish, the so-called Medusa's Head.

413

414 - *Biological Test*

415 It is usual with this kind of test to use particularly sensitive laboratory animals such as guinea pigs. The
416 inoculation of suspect material subcutaneously or intramuscularly is not recommended, especially when the
417 inoculate is full of secondary putrefactive bacteria. It is better to set up a test infection by coating the
418 material on an area of abdominal skin which had been previously shaved and scarified. This technique takes
419 advantage of the ability of *B. anthracis* to penetrate scarified skin, selecting it from the mixed microbial
420 flora. Rabbits die within 72 to 166 hours (Fasanella *et al.*, 2009) and this depends on the virulence of the
421 different strains of anthrax and the number of organisms. However, after a few hours a gelatinous,
422 haemorrhagic oedema forms at the point of inoculation, which is then followed by all the other
423 characteristics of an anthrax lesion.

424

425 - *Polymerase Chain Reaction (PCR)*

426 Polymerase Chain Reaction (PCR) is the method of choice as a parallel diagnostic test, whether performed
427 directly on clinical samples after non-selective enrichment of mixed cultures, or as a confirmation test for
428 suspect colonies. Additionally, PCR has become necessary for clarification of negative cultural results of
429 positive controls or when spiked agar plates are uninterpretable due to an overgrowth of *B. anthracis* by
430 haemolytic *Bacillus* spp. To confirm suspicious colonies, the DNA template for PCR can be extracted by

431 boiling resuspended colonies in TE buffer for ten minutes. To prepare DNA from a non-selective enrichment
432 culture, a DNA Preparation Kit generally gives a better result. DNA can be prepared directly from spores by
433 simple heating or autoclaving if very high numbers of spores ($>10^6$) are present. However, if only low
434 numbers of spores are present or expected, as in the case of nose swabs or in secondary contamination
435 scenarios, DNA preparation should be preceded by spore germination in culture. To identify virulent *B.*
436 *anthracis* strains, and for the differentiation of non-virulent strains, the presence of both plasmid pXO1
437 (toxins) and pXO2 (capsule formation) must be confirmed. Some chromosomal targets of rpoB, S-layer
438 protein genes and Ba813 very often lead to false-positive results from environmental samples
439 (Papaparaskevas *et al.*, 2004), while *plcR* is able to differentiate *Bacillus anthracis* from *Bacillus cereus* and
440 *Bacillus thuringiensis* (Easterday *et al.*, 2005).

441

442 - *Molecular characterization*

443 The full molecular characterization of *B. anthracis* was first completed with the Anthrax Genome Project,
444 which in 2003 led to the publication of the genome of strain *B. anthracis* Ames type (Read *et al.*, 2003). For
445 the scientific community this was a milestone, so that today, in Genbank there are 14 assembly, 5 complete
446 and 4 incomplete sequence projects of 19 strains of anthrax. The genome of *B. anthracis* is composed of one
447 chromosome with more than 5,000,000 bases and two plasmids, pXO1 with over 180,000 bases, and pXO2
448 with over 94,000 bases (Read *et al.*, 2003).

449

450 *B. anthracis* represents one of the most genetically homogeneous bacteria, with more than 99% of
451 homologous nucleotide sequences. Searches made before 2001 showed small molecular variations among
452 strains of different origin. In fact the complete sequence of the gene PagA (Protective Antigen) and the
453 MLST (Multiple Locus Sequencing Typing) analysis of housekeeping genes revealed a few mutations
454 (Okinaka *et al.*, 1999; Price, *et al.* 1999). The revealed genetic crystallization is a direct consequence of its
455 *modus vivendi*. In fact the vegetative form alternates with the spore form in an unpredictable frequency as a
456 consequence of the regional ecology of the disease such that it may take years or even decades between
457 infectious cycles. The evolutionary pressure is extremely low in the dormant (spore) stages of the cycle,
458 remaining minimal during the active phase (vegetative form) because it is estimated that a maximum of 40
459 bacterial generations can follow (Keim *et al.*, 2004). Consequently *B. anthracis* evolution is temporarily
460 reduced as the number of generations in a year are few when compared with *Escherichia coli* (Gutmann *et*
461 *al.*, 1994) The precise molecular typing of strains of *B. anthracis* is crucial both for forensic analyses of
462 biosecurity and bioterrorism events and for epidemiological investigations of natural outbreaks. The
463 traditional methods are not very recommended for anthrax genotyping because they are unable to detect
464 small genomic differences.

465

466 The genomic diversity is mainly the result of events during the evolution of the bacterium and genomic
467 analysis must rely on molecular markers as polymorphic as possible with a high rate of mutation. The

468 anthrax genotyping methods currently in use test different types of markers in relation to the utility of the
469 analysis.

470

471 The genotyping method, considered to have a low resolution, is the analysis of SNPs (Single Nucleotide
472 Polymorphisms) and identifies point mutations in the genome. These markers have a good stability with a
473 genomic mutation rate of 10^{-10} . While there is a low rate of mutation some 35000 SNPs comprise the entire
474 genome of anthrax (Pearson *et al.*, 2004, Read *et al.*, 2002). At present the opportunity to test all the
475 identified SNPs in any isolate is technically difficult and financially expensive. However some studies have
476 helped identify 12 canonical SNPs that are the most stable and homoplasmic and can be used for phylogenetic
477 investigations. Polymorphism analyses may be carried out using Snapshot or with real time PCR assays with
478 TaqMan MGB probes (Van Ert *et al.*, 2007)

479

480 The high resolution typing assay par excellence is that of Multiple Locus VNTR Analysis (MLVA) as it
481 seeks to identify specific genomic regions known as Variable Number Tandem Repeat (VNTR). These
482 regions of repeated DNA in tandem by their nature have a higher rate of mutation. The frequency of
483 mutation of these markers in *B. anthracis* is comparable to 10^{-5} with a high variability depending on the
484 locus (Keim *et al.*, 2004). This technique initially with 8 VNTR was able to identify 89 genotypes among 400
485 isolates from around the world (Keim *et al.*, 2000), while the 15 VNTR assay increased this to 221
486 genotypes among 1033 isolates. The method has now been increased up to 25 loci (Lista *et al.*, 2006), which
487 allows an excellent discrimination. Technically, the VNTR are searched using capillary sequencers to
488 analyse DNA fragments.

489

490 Lately high resolution assays were reported that examine markers called SNRs (Single Nucleotide Repeats),
491 a sort of VNTR consisting of repeated sequences of polyA. Stratilo *et al.* (2006) through a bioinformatics
492 analysis identified specific regions with a mutation rate of 10^{-4} . Utilisation of these regions allows
493 discrimination between organisms with the same MLVA pattern and thus allows sub-genotyping. The
494 instability of these loci doesn't make them homoplasmic because back-mutations often occur. Their use can
495 differentiate strains within the same outbreak or epidemic. A recent study has suggested the use of a panel of
496 4 SNR markers (Kenefic *et al.*, 2008).

497

498 The described genotyping methods can be understood in a hierarchical way. The SNPs being at low-power of
499 discrimination can be used for phylogenetic investigations. On the other hand VNTRs and SNRs have high
500 discriminatory powers. The first for its high diversity and homoplasia is able to correctly define the
501 genotype, while the latter searching for any signs of redundancy is considered suitable for identifying sub-
502 genotypes. All the methods described are best performed by specialized laboratories experienced in
503 molecular biology (Keim *et al.*, 2004).

504

Vaccines

505

506 The veterinary vaccines currently used to fight anthrax are composed of spores from attenuated strains of *B.*
507 *anthracis*. They are classified into two categories (Turnbull P., 1991):

508 - Live attenuated vaccines, capsulated and atoxigenic cap⁺/tox⁻; e.g., Pasteur vaccine.

509 - Live attenuated vaccines, not capsulated and toxigenic cap⁻/tox⁺; e.g., Sterne and STI vaccines
510 (Turnbull, 1991a; Fasanella *et al.*, 2001).

511

512 *Pasteur vaccine*

513 The Pasteur vaccine belongs to the first group and it is characterized by a plasmid pattern pX01⁻/pX02⁺. The
514 inactivation of *B. anthracis* is a function of the growth conditions. Grown at 42°C for 21 days it produces an
515 attenuated strain, Pasteur vaccine type 1. It is not pathogenic for guinea pigs but it is pathogenic for mice.
516 .When grown at 42°C for 14 days it produces an attenuated strain that is more pathogenic than Pasteur
517 vaccine type 1. It is pathogenic for both guinea pigs and mice, but is not pathogenic for rabbits. Pasteur
518 vaccines should not produce toxic factors. Seroanalysis of goats vaccinated with Pasteur vaccine did not
519 show the production of specific antibodies (unpublished data). Pasteur vaccines were used in the past, and
520 they routinely produced a 3% mortality. Presently only a few countries still use this vaccine. In practice, the
521 preparations used were “hot”, containing a mix of cap⁺/tox⁻ and cap⁺/tox⁺ organisms, thus the mortality
522 noted historically can be explained in this way.

523

524 *Sterne vaccine*

525 The Sterne vaccine represents another group of vaccines. It was produced for the first time in 1939, and was
526 obtained by cultivating *B. anthracis* on a medium with 50% horse serum at 30% CO₂ atmosphere for 24
527 hours. It is very protective. This and similar vaccines are characterized by an elevated protective capacity
528 and very low residual virulence. The attenuation of these strains is due to the loss of plasmid pX02 encoding
529 the capsule synthesis; the spores germinate in the vaccinated animals and sufficient non-encapsulated
530 vegetative forms germinate and produce toxic factors which are then neutralized by phagocytes allowing the
531 synthesis of sufficient toxin to stimulate the protective immunity, but not sufficient to damage the host.

532

533 The animal vaccines that use Sterne strain 34F₂ are formulated with approximately 10⁷ spores per ml
534 suspended in 0,5 ml 50% glycerine-saline. The protective effect of a single dose of strain 34F₂ vaccine is
535 expected to last about one year and an annual booster is recommended for livestock in endemic areas. Horses
536 and other equids can respond poorly and need two doses 4-8 weeks apart. South American camelids and
537 goats are very sensitive to Sterne and should be first vaccinated with a quarter-dose, followed 3-4 weeks later
538 by a full dose; the best procedure is that they are vaccinated in their tail fold. The duration of the protection
539 has never been systematically studied and is an area of research that needs to be studied in depth. Sterne
540 vaccine is less expensive compared to the recombinant vaccines and actually it represents the best vaccine
541 for routine anthrax control programs, especially if it is administered in the spring, to ensure the presence of
542 protective immunity during the summer months, when most anthrax outbreaks occur. Spring vaccination

543 should provide adequate protection in those countries where livestock is grazed on high pastures during the
544 summer but are exposed when coming down to their winter pastures at a lower altitude.

545

546 *Third group of Bacillus anthracis vaccines: Carbosap strain*

547 The strain “Carbosap” was obtained from Prof. Cilli’s research group in the “Istituto Vaccinogeno of
548 Asmara”, during World War II. It was used from 1949 up to 2006 in Italy to immunize cattle and sheep and
549 then it was replaced by the Sterne vaccine. The PCR analysis using specific primers for the genes encoding
550 for PA, LF, EF and capsule showed the presence of both plasmids, pXO1 and pXO2 (Fasanella *et al.*, 2001).
551 It has the same plasmid pattern of a pathogenic strain and shows no genetic difference in the DNA sequences
552 for the genes involved in the virulence (Adone *et al.*, 2002). Carbosap vaccine is pathogenic for mice and
553 guinea pigs but it is apathogenic for rabbits.

554

555 *Emerging Vaccines*

556 A recent recombinant vaccine, whose preparation requires expensive procedures, could be considered a
557 useful vaccine in cases of emergency or during an anthrax epidemic when it is necessary to induce high
558 levels of protective antibodies in a very short time. Used in conjunction with long-acting antibiotics
559 recombinant vaccines have the capacity to produce a rapid response at the same time as infections are
560 neutralized. (Fasanella *et al.*, 2008).

561

562

563 **Therapy**

564

565 Usually in most herbivores anthrax develops so quick that it is not always possible to implement a
566 successful treatment.

567

568 The therapy is successful if it is applied as soon as possible after the animal is diagnosed to be ill, because it
569 has to prevent the production of toxins and antibiotics have no effect on toxins. In practice, therapy is
570 effective only in the early stages of the disease if it doesn’t have an hyperacute course. Antibiotic therapy in
571 humans is important. The current standard for anthrax inhalation post exposure therapy is ciprofloxacin twice
572 a day for 60 days (<http://www.bt.cdc.gov/agent/anthrax/needtoknow.asp>) (Food and Drug Administration.
573 Prescription drug products; Doxycycline and Penicillin G Procaine administration for inhalational anthrax
574 (post-exposure). Federal Register 2001;66:55679) (Friedlander *et al.*, 1993). However in a study of 65
575 isolates of *B. anthracis* to determine the patterns of antimicrobial susceptibility it was found that one isolate
576 of *B. anthracis* was beta-lactamase positive and resistant to penicillin. All *B. anthracis* isolates were
577 susceptible to chloramphenicol, ciprofloxacin, clindamycin, rifampicin, tetracycline and vancomycin
578 (Mohammed *et al.*, 2002). In another study 22 *B. anthracis* isolates were tested for susceptibility to 27
579 antimicrobial agents by agar dilution. All isolates were sensitive to penicillins and did not produce beta-

580 lactamase. Although all isolates were sensitive to cefazolin, cephalothin, cephradine and cefoperazone, 19
581 isolates were resistant to cefuroxime, 18 to cefotaxime, 18 to ceftizoxime, 9 to ceftriaxone and 21 to
582 ceftazidime. All isolates were also found to be sensitive to ofloxacin and ciprofloxacin (Doğanay and Aydin,
583 1991). Coker and colleagues found that in a cluster-selective collection of 25 isolates 3/25 were resistant to
584 penicillin and 5 to cefuroxime. (Coker *et al.*, 2002) However, unpublished results from testing some 1200
585 isolates showed that 3% to 6% were resistant to penicillin depending also on the region in the world the
586 isolate came from. This applied to other antibiotics and to phage resistance. Antibiotic and phage resistance
587 is not unusual, it is just not common.

588

589 There are many antibiotic options to eliminate an anthrax infection, but there are no therapeutic options to
590 combat the LF-mediated toxemia and the tissue destruction during an ongoing infection or during the
591 residual toxemia that persists even after the bacteria have been eliminated by antibiotics. Research on
592 inhibitors of toxins has led to the identification of various substances whose action appears to be effective.
593 Green tea extracts are rich in polyphenols and some of them are good inhibitors of LF *in vitro* and *in vivo*
594 (Dell'Aica *et al.*, 2004). The best one is (-)epigallocatechin-3-gallate (EGCG). Branched peptides have been
595 found to inhibit both EF and LF binding to PA (Pini *et al.*, 2008). A powerful inhibitor of EF is adefovir
596 dipivoxil (Shen *et al.*, 2004). Using a rabbit model we tested EGCG and adefovir alone or in combination
597 with antibiotics to assess the possible therapeutic role of these potent inhibitors of both anthrax toxins. We
598 found that they are protective only if they are given in the very beginning of the infection (Fasanella and
599 Tonello, unpublished). This suggests that the role of toxins is critical for the initiation of pathology and that
600 therapy with toxin inhibitors may not be promising (Fasanella and Tonello, unpublished). However, it has
601 been demonstrated that a small molecule, hydroxamate LF inhibitor, can ameliorate the toxemia
602 characteristic of an active *B. anthracis* infection and might be a vital addition to our ability to combat
603 anthrax. (Shoop *et al.*, 2005). Finally, capsule-degrading enzymes favour the activity of neutrophils and other
604 components of innate immunity offering a new approach that may be effective against antibiotic and/or
605 vaccine-resistant strains. (Scorpio *et al.*, 2008).

606

607

608 **Human anthrax**

609

610 Man is usually resistant to acquiring infection, but when infected may show three different clinical forms: the
611 cutaneous, respiratory, and intestinal form.

612

613 The *cutaneous* form begins with the classic malignant pustule most often is localized to the face, neck, arms,
614 hands or legs. Most frequently this involves specific high risk occupations; i.e. farmers, butchers, tanners,
615 wool carders, shearers, and veterinarians. The most common exposure comes from skinning and butchering
616 cattle sick or dead from anthrax. At the point of entry of the germ – a pre-existing scratch - there is a skin

617 redness which turns into a papule. Characteristically this lesion area is not painful. The surrounding area
618 appears hyperaemic and oedematous. The papule develops into vesicles that spontaneously or from
619 scratching break, and eventually it is covered with a black eschar. Sometimes the regional lymph nodes can
620 be inflamed. Cutaneous anthrax is easily treatable with antibiotics of choice, but if a pustule is neglected it
621 may evolve into a fatal septicemia. Some 10% of untreated cutaneous cases may die.

622

623 *Intestinal* anthrax results from the consumption of contaminated meat. Its symptoms include nausea, loss of
624 appetite, vomiting, and fever followed by abdominal pain, vomiting of blood, severe diarrhea, lesions and
625 soreness in the throat, difficulty swallowing, marked swelling of the neck and regional lymph glands.
626 Intestinal anthrax results in death in 25 percent to 60 percent of cases (Beatty *et al.*, 2003). The intestinal
627 form occurs less frequent and occurs in those developing countries where food safety controls measures are
628 de facto non-existent. A recent case occurred in an hamlet in Vietnam, where two families shared beef from
629 their two cows, which had died of an unknown cause. Those eating the meat started vomiting, complaining
630 of stomachache and suffering swollen legs. Two of them died in coma after being hospitalized
631 (<http://www.promedmail.org>). Thorough cooking will kill the vegetative cells and prior exposure does
632 provide some immunity so the attack rate in any incident is variable.

633

634 The *respiratory or pulmonary* form is the major cause of atypical haemorrhagic pneumonia starting with flu-
635 like symptoms, characterized by fever, muscle pains, coughing, red nose, and bloody sputum. Untreated
636 cases are fatal. A study on the first ten confirmed cases of inhalational anthrax caused by an intentional
637 release of *B. anthracis* in the United States showed that the median incubation period from the time of
638 exposure to onset of symptoms was 4 days (range 4 to 6 days). Symptoms at initial presentation included
639 fever or chills, sweats, fatigue or malaise, a minimal or non-productive cough, dyspnoea, and nausea or
640 vomiting. All ten patients had abnormal chest X-rays; abnormalities included infiltrates, pleural effusion, and
641 mediastinal widening. Computer tomography of the chest was performed on eight patients, and mediastinal
642 lymphadenopathy was present in seven (Jernigan *et al.*, 2001). Forty-one cases of documented inhalational
643 anthrax from the Sverdlovsk epidemic of 1979 showed that the lesions that were the most severe and
644 apparently of the longest duration were in the mediastinal lymph nodes and mediastinum (Meselson *et al.*,
645 1994). There and elsewhere, peripheral transudate surrounded a fibrin-rich oedema; necrosis of arteries and
646 veins was the most likely source of large haemorrhages displacing tissue or infiltrating tissues, respectively;
647 and apoptosis of lymphocytes was observed. Respiratory function was compromised by mediastinal
648 expansion, large pleural effusions, and haematogenous and retrograde lymphatic vessel spread of *B.*
649 *anthracis* into the lungs with consequent pneumonia. The central nervous system and intestines manifested
650 similar haematogenous spread, vasculitis, haemorrhages, and oedema. (Grinberg *et al.*, 2001.)

651

652

653 **Considerations**

654

655 *B. anthracis* is known for its rapid proliferation and dissemination in receptive hosts but we still know very
656 little about its ability to replicate outside the animal. Recently it was shown that *B. anthracis* can survive as
657 a saprophyte outside the host. The data suggest that horizontal gene transfer in the rhizosphere of grass
658 plants may play a role in the evolution of the *B. cereus* group species (Saile and Koehler, 2006). These data
659 were obtained under experimental but sterile conditions and we do not know if this actually occurs in nature.
660 It is clear that in the environment, where conditions are less favorable to the survival of vegetative forms, *B.*
661 *anthracis* spores are one of the most advanced forms of resistance known in nature. In the Kruger National
662 Park (Africa) *B. anthracis* spores have been isolated from animal bones estimated to be about 200 years old
663 (Smith *et al.*, 2000). The calcium cation is the most important component in the process of germination or in
664 the maintenance of latency and hence the calcareous soils, rich in calcium and typically with an alkaline pH
665 contribute to the formation of much more resistant spores. The persistence of spores in the ground is
666 essential but not in itself sufficient to give rise to new anthrax outbreaks. Van Ness, whose studies represent
667 a milestone in understanding the ecology of anthrax, noted that the outbreaks of anthrax develop mainly
668 during the dry months that follow a prolonged period of rain. These climatic aspects and the fact that the
669 spores are characterized by a high floating capacity suggest that water plays an important role in the ecology
670 of the bacterium. Rainwater, having washed away the surrounding ground, tends to stagnate in the low lying
671 parts favoring the concentration of spores. This sequence of events encourages the adhesion and the
672 distribution of spores on soil humus, so the chances to infect herbivores increase. However it takes time and
673 special natural events to create sites of concentrations of spores which can cause new infections in grazing
674 animals (Van Ness, 1971). Most of the existence of *B. anthracis* is held in the ground as spores until the ideal
675 conditions are created for its reproductive cycle that occurs in a different habitat, primarily domestic and
676 wild ruminants. Nature provides few opportunities to the bacterium for its replicative cycle, and the
677 development of an exceptional pathogenicity is the effective strategy aimed to significantly increase the
678 probability of success against the host's immune mechanisms. Rapid intense multiplication by the vegetative
679 cells quickly take the host to death.

680

681 Although many of the new generations of bacteria will be neutralized by putrefactive processes, a good part
682 survives and spreads in the surrounding soil as spores, ensuring the standard of environmental density of the
683 bacteria that is an essential condition for the continuation of the species. In summary, the few cases of
684 anthrax that occur each year are merely the result of a natural ecological balance that seeks through these
685 extraordinary events simply to promote the maintenance of a bacterial species that otherwise would have
686 been extinguished some time ago.

687

688 Animals from areas free of anthrax and moved to risk areas are more susceptible to disease. The project to
689 reintroduce red deer (*Cervus elaphus*) into some nature reserves in Basilicata (Southern Italy), is facing
690 major obstacles just because of the sensitivity of this particular animal species to anthrax infection (Fasanella

691 *et al.*, 2007). At this time we do not know if this is related to the specific animal species or to a lack of
692 natural antibodies, but it is certain they come from ecosystems in which anthrax was not present. In nature
693 there are no natural self-destructive behaviors and every living being has evolved its own strategies for
694 survival, not only in terms of preservation of their species but also in that of its ecosystem. So could we not
695 hypothesize that *B. anthracis* has a protective role in the delicate balance of its ecosystem, somehow
696 preserving animal species that are an integral part of that particular area from the possible risk of extinction
697 from infectious diseases introduced by previously unknown animals from different environments? (personal
698 opinion)

699
700 The control and eradication programs of this disease involves livestock vaccination and the correct
701 management of infected carcasses. Incineration *in situ* is the best method to reduce the contamination level
702 of soil, but it is not always possible. A major constraint is the low budget that civil authorities assign for the
703 control of this disease. This makes it impossible to use the most cost effective technologies for control
704 programs. A global responsibility should oblige local governments to guarantee sufficient budgets for control
705 and eradication programs of this disease and not just for emergency situations.

706

707 **Conflict of Interest Statement**

708 None.

709

710 **References**

711 Abrami, L., Reig, N., Van der Goot, F.G., 2005. Anthrax toxin: the long and winding road that leads to the
712 kill. Trends Microbiol. 13, 72-78.

713 Adone, R., Pasquali, P., La Rosa, G., Marianelli, C., Muscillo, M., Fasanella, A., Francia, M., Ciuchini, F.,
714 2002. Sequence analysis of the genes encoding for the major virulence factors of Bacillus anthracis vaccine
715 strain 'Carbosap'. J. Appl.Microbiol. 93, 117-21.

716 Beatty, M.E., Ashford, D.A., Griffin, P.M., Tauxe, R.V., Sobel, J., 2003. Gastrointestinal Anthrax: Review
717 of the Literature. Arch. Intern. Med. 163, 2527-2531.

718 Blackburn, J.K. 2006. Evaluating the spatial ecology of anthrax in North America: examining
719 epidemiological components across multiple geographic scales using a GIS-based approach, Doctoral
720 dissertation, Louisiana State University, pp141.

721 Blancou, J., 2003. History of the surveillance & control of transmissible animal diseases. OIE, 2003

- 722 Boyden, E. D., Dietrich, W.F., 2006. Nalp1b controls mouse macrophage susceptibility to anthrax lethal
723 toxin. *Nat. Genet.* 38, 240-244.
- 724 Coker, P.R., Smith, K.L., & Hugh-Jones, M.E., 2002. Antimicrobial susceptibilities of diverse *Bacillus*
725 *anthracis* isolates. *Antimicrobial Agents & Chemotherapy* 2002 Dec. 46(12), 3843-3845.
- 726 Coker, P.R., Smith, K.L., Fellows, P.F., Rybachuck, G., Kousoulas, K.G., & Hugh-Jones M.E., 2003
727 *Bacillus anthracis* virulence in guinea pigs vaccinated with anthrax vaccine adsorbed is linked to plasmid
728 quantities and clonality. *Journal of Clinical Microbiology.* 41 (3), 1212-1218.
- 729 Collier, R.J., Young J. A., 2003. Anthrax toxins. *Annu. Rev. Cell. Dev. Biol.* 19, 45-70.
- 730 Contini, A., 1995. *Bacillus*. In: Andreani, E., Buonavoglia, C., Compagnucci, M., Contini, A., Farina, R.,
731 Flammini, C., Gentile, G., Gualandi, G., Mandelli, G., Panina, G., Papparella, V., Pascucci, S., Poli, G.,
732 Redaelli, G., Ruffo, G., Scatozza, F., Sidoli, L., *Malattie infettive degli animali*, UTET, Torino, 20, 290-
733 295.
- 734 Dal Molin, F., Tonello, F., Ladant, D., Cornetta, I., Zamparo, I., Di Benedetto, G., Zacco, M., Montecucco,
735 C., 2006. Cell entry and cAMP imaging of anthrax edema toxin. *EMBO J.* 25, 5405-5413.
- 736 Davies, J.C., 1983. A major epidemic of anthrax in Zimbabwe. Part II. *Cent Afr J Med* 29(1), 8-12.
- 737 Dell'Aica, I., Donà, M., Tonello, F., Piris, A., Mock, M., Montecucco, C., Garbisa, S., 2004. Potent inhibitors
738 of anthrax lethal factor from green tea. *EMBO Rep.* 5(4), 418-422.
- 739 Doğanay, M., Aydin, N., 1991. Antimicrobial susceptibility of *Bacillus anthracis*. *Scand J Infect Dis.*
740 23(3), 333-5
- 741 de Vos, V., 1994. Anthrax. In: Coetzer J., Thomson G.R., Tustin R., Kriek N., (eds.) *Infectious diseases of*
742 *livestock*, volume II, Oxford University Press. 153, 1262-1289.
- 743 Easterday, W.R., Van Ert, M.N., Simonson, T.S., Wagner, D.M., Kenefic, L.J., Allender, C.J., Keim, P.,
744 2005. Use of Single Nucleotide Polymorphisms in the *plcR* Gene for Specific Identification of *Bacillus*
745 *anthracis*. *J Clin Microbiol.* 2005 April. 43(4): 1995-1997.
- 746 Ebedes, H., 1976. Anthrax epizootics in Etosha National Park. *Modoqua*, 10, 99-118.
- 747 Fasanella, A., Losito, S., Trotta, T., Adone, R., Massa, S., Ciuchini, F., Chiocco, D., 2001. Detection of
748 anthrax vaccine virulence factors by polymerase chain reaction. *Vaccine.* 19, 4214-4218.
- 749 Fasanella, A., Van Ert, M., Altamura, S.A., Garofolo, G., Buonavoglia, C., Leori, G., Huynh, L., Zanecki, S.,
750 Keim, P., 2005. Molecular diversity of *Bacillus anthracis* in Italy. *J Clin Microbiol.* 43, 3398-3401.

- 751 Fasanella, A, Palazzo, L, Petrella, A, Quaranta, V, Romanelli, B, Garofolo, G., 2007. Anthrax in red deer
752 (*Cervus elaphus*), Italy. *Emerg Infect Dis.*, 13(7), 1118-9
- 753 Fasanella, A., Tonello, F., Garofolo, G., Muraro, L., Carattoli, A., Adone, R., Montecucco, C., 2008.
754 Protective activity and immunogenicity of two recombinant anthrax vaccines for veterinary use. *Vaccine.*
755 26(45), 5684-8
- 756 Fasanella, A., Scasciamacchia, S., Garofolo, G. 2009. The behaviour of virulent *Bacillus anthracis* strain
757 AO843 in rabbits. *Vet Microbiol.* 133(1-2), 208-9.
- 758 Ferguson, L. C., 1981: Anthrax. In: Leman A. D., Glock R. D., Mengeling W. L., Penny R. C. H., Scholl E.,
759 Straw B.(eds). *Diseases of Swine.* 5th edn. Ames, Iowa: Iowa State University Press
- 760 Fouet, A., Smith, K.L., Keys, C., Vaissaire, J., Le Doujet, C., Lévy, M., Mock, M., Keim, P., 2002. Diversity
761 Among French *Bacillus anthracis* Isolates. *J Clin Microbiol.* 40, 4732–4734.
- 762 Food and Drug Administration. Prescription drug products; Doxycycline and Penicillin G Procaine
763 administration for inhalational anthrax (post-exposure). *Federal Register* 2001;66:55679;
- 764 Friedlander, A.M., Welkos, S.L., Pitt, M.L., Ezzell, J.W., Worsham, P.L., Rose, K.J., Ivins, B.E., Lowe, J.R.,
765 Howe, G.B., Mikesell, P., et al., 1993. Postexposure prophylaxis against experimental inhalation anthrax. *J*
766 *Infect Dis.* 167, 1239-43.
- 767 Gleiser, C.A., 1967. Pathology of anthrax infection in animal hosts. *Federation Proceedings*, 26. 1518-
768 1521.
- 769 Grinberg, L.M., Abramova, F.A., Yampolskaya, O.V., Walker, D.H., Smith, J.H., 2001. Quantitative
770 pathology of inhalational anthrax I: quantitative microscopic findings. *Mod Pathol.* 14(5), 482-95
- 771 Gutmann, D.S., Dykhuizen, D.E., 1994. Clonal divergence in *Escherichia coli* as a result of recombination,
772 not mutation. *Science.* 266, 1380-1383.
- 773 Henning, M.W., 1956. Anthrax. In: *Animal diseases in South Africa.* 3rd ed. South Africa: Central News
774 Agency Ltd.
- 775 Hudson, M.J., Beyer, W., Böhm, R., Fasanella, A., Garofolo, G., Golinski, R., Goossens, P.L., Hahn, U.,
776 Hallis, B., King, A., Mock, M., Montecucco, C., Ozin, A., Tonello, F., Kaufmann, S.H., 2008. *Bacillus*
777 *anthracis*: balancing innocent research with dual-use potential. *Int J Med Microbiol.* 298(5-6), 345-64.
- 778 Hugh-Jones, M.E. 1999. 1996-97 Global Anthrax report. *J Appl Microbiol.* 87, 189-191.
- 779 Hugh-Jones, M.E, de Vos, V. 2002. Anthrax and wildlife. *Rev. Sci. Tech.* 2, 359-389.

- 780 Hugh-Jones, M.E., Blackburn, J. 2009. The ecology of *Bacillus anthracis*. Molecular Aspects of Medicine.
781 *in press*
- 782 Jackson, P.J., Hugh-Jones, M.E., Adair, D.M., Green, G., Hill, K.K., Kuske, C.R., 1998. PCR analysis of
783 tissue samples from the 1979 Sverdlovsk anthrax victims: the presence of multiple *Bacillus anthracis* strains
784 in different victims. Proc. Natl. Acad. Sci. 95, 1224–1229.
- 785 Jernigan, D.B., Raghunathan, P.L., Bell, B.P., Brechner, R., Bresnitz, E.A., Butler, J.C., 2002. Investigation
786 of bioterrorism-related anthrax, United States, 2001: epidemiologic findings. Emerg. Infect. Dis. Vol. 8,
787 1019–1028.
- 788 Jernigan, J.A., Stephens, D.S., Ashford, D.A., Omenaca C., Topiel M.S., Galbraith M., Tapper M., Fisk T.L.,
789 Zaki S., Popovic T., Meyer R.F., Quinn C.P., Harper S.A., Fridkin S.K., Sejvar J.J., Shepard C.W.,
790 McConnell M., Guarner J., Shieh W.J., Malecki J.M., Gerberding J.L., Hughes J.M., Perkins B.A., 2001.
791 Anthrax Bioterrorism Investigation Team Bioterrorism-related inhalational anthrax: the first 10 cases
792 reported in the United States. Emerg Infect Dis. 7(6), 933-944.
- 793 Keim, P., L.B. Price, A.M. Klevytska A.M., K.L. Smith, J.M., Schupp, R. Okinaka, Jackson P. and M.E.
794 Hugh Jones, 2000. Multiple-Locus Variable-Number Tandem Repeat Analysis Reveals Genetic
795 Relationships within *Bacillus anthracis*. *J. Bacteriology*. 182, 2928-2936.
- 796 Keim, P., Van Ert, M.N., Pearson, T., Vogler, A.J., Huynh, L.Y., Wagner, D.M., 2004. Anthrax molecular
797 epidemiology and forensics: using the appropriate marker for different evolutionary scales. Infect. Genet.
798 Evol. 4, 205-213.
- 799 Kenefic, L.J., Beaudry, J., Trim, C., Huynh, L., Zanecki, S., Matthews, M., Schupp, J., Van Ert, M., Keim, P.
800 2008. A high resolution four-locus multiplex single nucleotide repeat (SNR) genotyping system in *Bacillus*
801 *anthracis*. J Microbiol Methods. Jun:73(3), 269-72.
- 802 Koch, R. 1876. Beitrage zur Biologie der Pflanzen. In Cohn, F. (Ed.). Zweites Heft, Breslau, 277-310.
- 803 Kraneveld, F.C., Djaenoedin, R. 1940. Test on the dissemination of anthrax by *Tabanus rubidus* in horses
804 and buffalo. Overgedrukt uit de Nederlands-Indische Bladen Voor Diergeneeskunde. 52, 339-80.
- 805 Kreidl, P., Stifter, E., Richter, A., Aschbachert, R., Nienstedt, F., Unterhuber, H., Barone, S., Huemer, H.P.,
806 Carattoli, A., Moroder, L., Ciofi Degli Atti, M.L., Rota, M.C., Morosetti, G., Larcher, C., 2006. Anthrax in
807 animals and a farmer in Alto Adige, Italy. Euro Surveill. 11(7).
- 808 Leendertz, F.H., Ellerbrok, H., Boesch, C., Couacy-Hymann, E., Matz-Rensing, K., Hakenbeck, R.,
809 Bergmann, C., Abaza, P., Junglen, S., Moebius, Y., Vigilant, L., Formenty, P., Pauli, G. 2004. Anthrax kills
810 wild chimpanzees in a tropical rainforest. Nature. 430, 451-452.

- 811 Leppa, S.H., 1982. Anthrax toxin edema factor: a bacterial adenylate cyclase that increases cyclic AMP
812 concentrations of eukaryotic cells. *Proc. Natl. Acad. Sci. USA.* 79, 3162-3166.
- 813 Lista, F., Faggioni, G., Valjevac S., Ciammaruconi, A., Vaissaire, J., le Doujet, C., Gorge, O., de Santis, R.,
814 Carattoli, A., Ciervo, A., Fasanella, A., Orsini, F., D'Amelio, R., Pourcel, C., Cassone, A., Vergnaud, G.
815 2006. Genotyping of *Bacillus anthracis* strains based on automated capillary 25-loci multiple locus variable-
816 number tandem repeats analysis. *BMC Microbiology.* 6-33.
- 817 Marcato, P.S., 1981: *Anatomia e istologia patologica speciale dei mammiferi domestici*, Edagricole, Bologna
- 818 Meselson, M., Guillemin, J., Hugh-Jones, M.E., Langmuir, A., Popova, I., Shelokov, A., Yampolskaya, O.,
819 1994. The Sverdlovsk anthrax outbreak of 1979. *Science*, Nov 18. 266(5188), 1202-8.
- 820 Mitzmain, M.B., 1914. Experimental insect transmission of anthrax. *Public Health Reports.* 29, 75-7716.
- 821 Mohammed, M.J., Marston, C. K., Popovic, T., Weyant, R.S, and Tenover, F.C., 2002. Antimicrobial
822 Susceptibility Testing of *Bacillus anthracis*: Comparison of Results Obtained by Using the National
823 Committee for Clinical Laboratory Standards Broth Microdilution Reference and Etest Agar Gradient
824 Diffusion Methods. *J. Clin. Microbiol.* 40(6), 1902-1907.
- 825 Morris, H., 1918. Blood-sucking insects as transmitters of Anthrax or Charbon, *Louisiana Bulletin* No. 163,
826 March, 1918, pp15.
- 827 Nishi, J.S., Dragon, D.C., Elkin, B.T., Mitchell, J., Ellsworth, T.R., Hugh-Jones, M.E., 2002 Emergency
828 response planning for anthrax outbreaks in bison herds of northern Canada: A balance between policy and
829 science. *Ann. N. Y. Acad. Sci.* 969, 245-50.
- 830 Okinaka, R.T., Cloud, K., Hampton, O., Hoffmaster, A.R., Hill, K.K., Keim, P., Koehler, T.M., Lamke, G.,
831 Kumano, S., Mahillon, J., Manter, D., Martinez, Y., Ricke, D., Svensson, R., Jackson, P.J. 1999. Sequence
832 and organization of pXO1, the large *Bacillus anthracis* plasmid harboring the anthrax toxin genes. *J*
833 *Bacteriology.* 181(20), 6509-6515.
- 834 Papaparaskevas, J., Houhoula, D.P., Papadimitriou, M., Saroglou, G., Legakis, N.J., Zerva, L. 2004. Ruling
835 out *Bacillus anthracis*. *Emerg. Infect. Dis.* 10, 732-735.
- 836 Pearson T, Busch JD, Ravel J, Read TD, Rhoton SD, U'Ren JM, Simonson TS, Kachur SM, Leadem RR,
837 Cardon ML, Van Ert MN, Huynh LY, Fraser CM, Keim P. 2004. Phylogenetic discovery bias in *Bacillus*
838 *anthracis* using single-nucleotide polymorphisms from whole-genome sequencing. *Procl Nat Acad Sci*
839 *USA.* 101(37), 13536-13541.
- 840 Pienaar, U.D., 1967. Epidemiology of anthrax in wild animals and the control of epizootiocs in the Kruger
841 National Park, South Africa. *Fed Prot.* 26, 1496-1502.

- 842 Pini, A., Brunetti, J., Falciani, C., Fabbrini, M., Bracci, L., 2008. Solubility improvement of an anthrax toxin
843 peptide inhibitor by rational amino acid randomization. *Protein Pept Lett.* 15(6), 562-566.
- 844 Price, L.B., Hugh-Jones, M.E., Jackson, P.J., Keim, P., 1999. Genetic diversity in the protective antigen gene
845 of *Bacillus anthracis*. *J. Bacteriology.* 181, 2358-2362.
- 846 Rao, N.S., Mohiyudeen S., 1958. Tabanus flies as transmitters of anthrax – a field experience, 35, 348-353.
- 847 Read, T. D., Peterson, S. N., Tourasse, N., Baillie, L. W., Paulsen, I. T., Nelson, K. E., Tettelin, H., Fouts,
848 D., Eisen, J. A., Gill, S. R., Holtzapple, E. K., Økstad, O. A., Helgason, E., Rislstone, J., Wu, M., Kolonay, J.
849 F., Beanan, M. J., Dodson, R. J., Brinkac, L. M., Gwinn, M., DeBoy, R. J., Madpu, R., Daugherty, S. C.,
850 Durkin, A. S., Haft, D. H., Nelson, W. C., Peterson, J. D., Pop, M., Khouri, H. M., Raduna, D., Benton, J. L.,
851 Mahamoud, Y., Jiang, L., Hance, I. R., Weidman, J. F., Berry, K. J., Plaut, R. D., Wolf, A. W., Watkins, K.
852 L., Nierman, W. C., Hazen, A., Cline, R., Redmond, C., Thwaite, J. E., White, O., Salzberg, S. L.,
853 Thomason, B., Friedlander, A. M., Koehler, T. M., Hanna, P. C., Kolstø, A.-B., and Fraser, C. M., 2003. The
854 genome sequence of *Bacillus anthracis* Ames and comparison to closely related bacteria. *Nature.* 423,81–86.
- 855 Read, T.D., Salzberg, S.L., Pop, M., Shumway, M., Umayam, L., Jiang, L., Holtzapple, E., Busch, J.D.,
856 Smith, K.L., Schupp, J.M., Solomon, D., Keim, P., Fraser, C.M., 2002. Comparative genome sequencing for
857 discovery of novel polymorphisms in *Bacillus anthracis*. *Science.* 296(5575), 2028-2033.
- 858 Saile, E., Koehler, T.M., 2006. *Bacillus anthracis* multiplication, persistence, and genetic exchange in the
859 rhizosphere of grass plants. *Appl Environ Microbiol.* 72(5), 3168-3174.
- 860 Scobie, H.M., Young, J.A., 2005. Interactions between anthrax toxin receptors and protective antigen. *Curr*
861 *Opin Microbiol.* 8(1), 106-112.
- 862 Scorpio, A., Tobery S.A., Ribot W.J., Friedlander A.M., 2008. Treatment of Experimental Anthrax with
863 Recombinant Capsule Depolymerase. *Antimicrobial Agents and Chemotherapy.* Vol. 52(3), 1014-1020.
- 864 Shen, Y., Zhukovskaya, N.L., Zimmer, M.I., Soelaiman, S., Bergson, P., Wang, C.R., Gibbs, C.S. & Tang,
865 W.J. 2004. Selective inhibition of anthrax edema factor by adefovir, a drug for chronic hepatitis B virus
866 infection. *Proc. Natl. Acad. Sci.* 101, 3242-3247.
- 867 Shoop, W.L., Xiong, Y., Wiltsie, J., Woods, A., Guo, J., Pivnichny, J.V., Felcetto, T., Michael, B.F., Bansal,
868 A., Cummings., R.T., Cunningham, B.R., Friedlander, A.M., Douglas, C.M., Patel, S.B., Wisniewski, D.,
869 Scapin, G., Salowe, S.P., Zaller, D.M., Chapman, K.T., Scolnick, E.M., Schmatz, D.M., Bartizal, K.,
870 MacCoss, M., Hermes, J.D., 2005. Anthrax lethal factor inhibition. *Proc Natl Acad Sci USA.* 102(22), 7958-
871 7963.

- 872 Smith, K.,L., De Vos, V., Bryden, H., Price, L.B., Hugh-Jones, M., Kleuytska, A., Price, L.B., Keim, P.,
873 2000. *Bacillus anthracis* diversity in Kruger National Park. J Clin Micro. Vol. 38(10), 3780-3784.
- 874 Smith, I.M., 1973. A brief review of Anthrax in domestic animals. Postgrad Med J. 49(574), 571-572.
875 Review.
- 876 Stein, C.D., 1947a. Some observations on the tenacy of *Bacillus anthracis*. Vet Med. 1947, 13-22.
- 877 Stein C.D., 1947b. Anthrax in animals and its relationship to the disease in man. Ann. NY Acad. Sci. 48,
878 507-534.
- 879 Sterne, M., 1959. Anthrax. In: Stableforth A. W. & Galloway I. A., (eds). Infectious Diseases of Animal.
880 Diseases due to Bacteria. Vol I. London: Butterworths Scientific Pubblcation.
- 881 Sterne, M., 1967: Distribution and economic importance of Anthrax. Fed Proc. 26(5),1493.
- 882 Stratilo, C.W., Lewis, C.T., Bryden, L., Mulvey, M.R., Bader, D., 2006. Single-nucleotide repeat analysis for
883 subtyping *Bacillus anthracis* isolates. J Clin Microbiol. 44(3), 777-778.
- 884 Tomaso, H., Bartling, C., Al Dahouk, S., Hagen, R.M., Scholz, H.C., Beyer, W., Neubauer, H., 2006.
885 Growth characteristics of *Bacillus anthracis* compared to other *Bacillus* spp. on the selective nutrient media
886 Anthrax Blood Agar and *Cereus* Ident Agar. Syst. Appl Microbiol. 29(1), 24-28.
- 887 Turell, M.J., Knudson, G.B., 1987. Mechanical transmission of *Bacillus anthracis* by stable flies (*Stomoxys*
888 *calcitrans*) and mosquitoes (*Aedes aegypti* and *Aedes taeniorhynchus*). Infect. Immun. 55, 1859-1961.
- 889 Turnbull, P.C.B., Barman, J.A., Lindeque, D.M., Joubert, F., Hubschle, O.J.B., Snoeyenbos, G.S., 1989.
890 Further progress in understanding anthrax in Etosha National Park. Mdoqua 16, 93-104;
- 891 Turnbull, P.C., 1991a. Anthrax vaccines: past, present and future. Vaccine. 1991 9(8), 533-539.
- 892 Turnbull, P.C., Bell, R.H., Saigawa, K., Munyenyembe, F.E., Mulenga, C.K., Makala, L.H. 1991b. Anthrax
893 in wildlife in the Luangwa Valley, Zambia. Vet. Rec. 17, 399-403.
- 894 Turnbull, P.C.,1998. Guidelines for the surveillance and control of Anthrax in human and animals.3rd edition.
- 895 Turner, A.J., Galvin, J.W., Rubira, R.J., Condron, R.J., Bradley, T. 1999. Experiences with vaccination and
896 epidemiological investigations on anthrax outbreak in Australia in 1997. J. Appl. Microbiol. 87, 294-297.
- 897 Uchida, I., Sekizaki, T., Hashimoto, K., Terakado, N., 1985. Association of the encapsulation of *Bacillus*
898 *anthracis* with a 60 megadalton plasmid. J.Gen.Microbiol. 131, 363-67.

- 899 Van Ert, M., Easterday, W.R., Huynh, L.Y., Okinaka, R.T., Hugh-Jones, M.E., Ravel, J., Zanecki, S.R. ,
900 Pearson, T., Simonson, T.S., U'Ren, J.M., Kachur SM, Leadem-Dougherty RR, Rhoton SD, Zinser G, Farlow
901 J, Coker PR, Smith KL, Wang B, Kenefic, L.J., Fraser-Liggett, C.M., Wagner, D.M., Keim, P. 2007. Global
902 Genetic Population Structure of *Bacillus anthracis*. PlosOne. 5, 461-471.
- 903 Van Ness, G.B., 1971. Ecology of anthrax. Science. Vol. 172 (990), 1303-1307.
- 904 Vitale, G., Bernardi, L., Napolitani, G., Mock, M., Montecucco, C., 2000. Susceptibility of mitogen-
905 activated protein kinase family members to proteolysis by anthrax lethal factor. Biochem. J. 352, 739-745.
- 906 Whitford, H.W., 1979: Anthrax. In: Steele J.K. (ed.). CRC Handbook Series in Zoonoses, Section A.:
907 Bacterial, Rickettsial and Mycotic Diseases. Vol I. Boca Raton, Florida: CRC Press, Inc.;
- 908

Figure 1: *Bacillus anthracis*. Vegetative form, Gram stain

Figure 2: *Bacillus anthracis*. Spore form, Shöeffler Fulton stain

Figure 3: *Bacillus anthracis* colonies in semi selective agar (TSPB). It can notice the typical aspect with indented margins.

Figure 4: *Bacillus anthracis* grown in liquid agar. The vaccine strain Sterne 34F2 grows mudding the medium, while the pathogenic strain creating flocculus.

Figure 5: Red deer found after a long time from Its death. The signs of wild carnivores are well evident

Figure 1



Figure 2

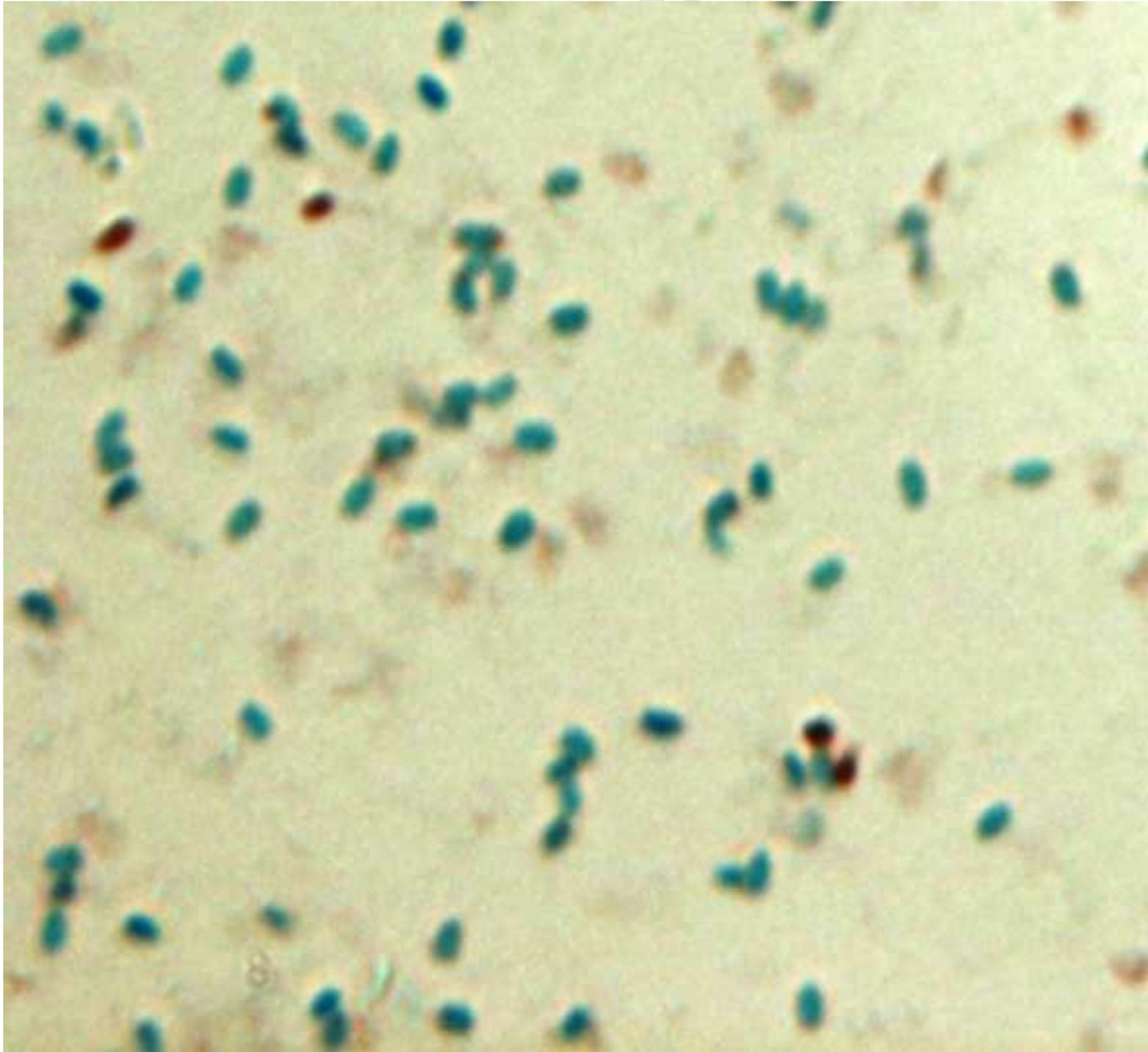




Figure 4

