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Corynebacterium diphtheriae in Skin Lesions in Ugandan Children

V. BEZJAK¹ & S.J. FARSEY²

Diphtheritic skin lesions are known to be relatively common in tropical as opposed to temperate climates, but the possible presence of Corynebacterium diphtheriae in skin lesions has not been investigated in East Africa, where classical diphtheria cases are rare. The authors therefore studied septic skin lesions and skin ulcers in 139 preschool children and schoolchildren in Uganda. A high percentage (up to 60%) of C. diphtheriae lesions was found, a majority of the isolations being of the mitis type. At the same time, low respiratory carrier rates for C. diphtheriae were found. The practical importance of the skin reservoir of C. diphtheriae for the development of specific diphtheria immunity in the tropics is stressed.

Soon after the introduction of active immunization programmes in most developed countries diphtheria started to decline rapidly and the disease has now practically disappeared from those countries. In a number of developing countries, however, diphtheria is still a problem, although in others respiratory diphtheria is rare or unknown despite the absence of immunization procedures. The latter countries are mostly in the tropics or subtropics -Nigeria (Ikejiani, 1961), Uganda (Bwibo, 1969), Solomon Islands (Liebow et al., 1946), Colombia (S. W. Bennett, personal communication) and the Marianas and Philippine Islands (Liebow et al., 1946). On the other hand, in some tropical countries -e.g., Madagascar (Saugrain, 1955) or Sudan (Hassan, 1968)-diphtheria is guite common and may even be very important.

In temperate areas, skin diphtheria is comparatively rare as a form of primary infection with *Corynebacterium diphtheriae*, and it is seldom diagnosed nowadays in such regions (Sviridenko, 1956; Wilson & Toshach, 1957; Funt, 1961; Belsey et al., 1969), but it appears to be important and frequent in the tropics and subtropics, especially as a secondary infection of septic skin lesions (Craig, 1919; Liebow et al., 1946; Bacon & Marples, 1955; Markham & Stenhouse, 1959; Tan Eng Tie, 1965; Marples, 1965; Bennett, 1968; Gunatillake & Taylor, 1968). It has not yet been determined what factor or factors cause C. *diphtheriae* skin infections to be so frequent in tropical areas.

MATERIALS AND METHODS

Patients

The patients studied were drawn from three different groups of institutions: (a) the Kasangati Health Centre out-patient clinic (15 km from Kampala), (b) the Oruchinga Ruanda Refugee Settlement School in Ankole District (south-western Uganda), and (c) three primary schools (Amudat, Moruita, Nakapiripirit) in Karamoja District (northeastern Uganda).

The numbers of patients in each group, their age, sex, tribal origin and type of skin lesions, are shown in Table 1.

The children with skin lesions from Kasangati included in the survey were those seen during weekly visits in the period from July 1968 to February 1969. The Ruanda refugee school at Ankole was surveyed on three occasions: in July and October 1968 and in January 1969. The schools in Karamoja were visited in March 1969 when testing histoplasmin sensitivity (Bezjak & Farsey, 1970a); all pupils with skin ulcers were included in the present study.

In the Kasangati survey it was not possible to get accurate information on diphtheria immunization, although it is likely that the children under 5 years

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Survey	No. of	Age group			Tribal origin	Type of skin lesion
	children	(years) Male	Female			
Kasangati Health Centre	40 ^a	0–18 ^b	23	17	26 Baganda, 14 others	Mostly impetigo
Ankole refugee school	54	5–18	40	14	53 Ruanda, 1 Nyankole	Ulcers only
Karamoja primary schools	35	5–18	35	0	10 Didinga, 6 Pokot, 19 others	Ulcers only
Total	129	0–18	98	31		

TABLE 1 PERSONAL DATA ON THE PATIENTS STUDIED

^a Data are not available for 10 other children studied at the Kasangati Health Centre.

^b 50% of patients were under 4 years of age.

had been previously immunized. In the other two surveys none of the children over 4 years had been immunized, as the national immunization programme in Uganda did not start until 1965.

Classification of skin lesions

The skin lesions seen in the three surveys could be classified as: (a) impetigo, (b) suspected diphtheritic ulcers, (c) tropical ulcers. To avoid any possible misunderstanding, the main characteristics of the lesions seen are described in detail.

Impetigo. The initial lesions (vesicles or bullae) ruptured, leaving superficial erosions, usually covered by purulent crusts. The crusts were thin or thick, varying in colour from yellow to brown or dirty black. The lesions were commonly seen on the limbs and face, sometimes on the head and neck or trunk. After healing they usually left no scar.

Suspected diphtheritic ulcers. These lesions started spontaneously as vesicles and then rapidly ulcerated, but the ulcers remained small (1 cm-2 cm) and took weeks to heal. The ulcers were well demarcated, the surrounding skin was congested, the edges were raised and undermined. The surface bled easily when swabbed. Some of them were covered with a dark crust. In the majority of children the ulcers were single, usually situated on the legs. This description is consistent with the second developmental stage of diphtheria ulcer given by Craig (1919). In our survey we did not see the " punched-out " ulcers described by several authors. Tropical ulcers. These started either as traumatic or as spontaneous vesicles with sero-sanguinous fluid; they later ruptured, exposing a stinking slough. These ulcers usually extended rapidly and were round or oval and deep, exposing the underlying muscle, tendon and other tissues. On healing, an unsightly scar was usually left behind. No Buruli ulcers (Clancey et al., 1961) were seen in any of the areas visited.

Bacteriological examination

Whether multiple or not, only one lesion in each patient was examined for *C. diphtheriae*. Usually a swab was taken and immediately inoculated on to half a plate (in the Ankole study) or a whole plate (Kasangati study) of tellurite-blood-agar. Media were incubated shortly afterwards (Kasangati study) or after a delay of 2-3 days. In the Karamoja survey charcoal swabs were taken and kept for 2-3 days in Stuart's medium before inoculation of tellurite-blood-agar plates; media were incubated immediately afterwards.

After 2 days of incubation at 37°C the telluriteblood-agar plates were examined and any suspected *Corynebacterium* colonies isolated. Subcultures were studied morphologically (microscopy and appearance of blood-agar cultures) and biochemically (glucose, sucrose, starch and nitrate), identified and typed. Atypical *C. diphtheriae* strains corresponded to the *mitis* type except for being nonhaemolytic. Toxigenicity tests were made in guinea-pigs by the intradermal protection technique.

RESULTS

A total of 139 Ugandan children with septic skin or skin ulcers were examined and in 69 (50%) C. diphtheriae was found. When divided into three groups according to the district of residence, the frequency of C. diphtheriae organisms varied between 32% (Kasangati) and 60% (Karamoja). The results are presented in Tables 2 and 3.

In Kasangati children 50 cases were examined, but detailed information was available only for 40 patients (Table 4). The majority (26) were children aged 2–15 years, and there were more boys than girls (23:17). In the majority of patients the lesions were on limbs (24), although there was not a single case with lesions on the upper limbs alone.

TABLE 2 C. DIPHTHERIAE IN SKIN LESIONS OF UGANDAN CHILDREN

Survey	No. of children examined	whose C. dipi	en from lesions h <i>theriae</i> lated	Type of skin lesions	
		No.	%		
Ankole refugee school	54	32	59	Ulcers only	
Karamoja (3 pri- mary schools)	35	21	60	Uicers only	
Kasangati Health Centre	50	16	32	Impetigo and ulcers	
Total	139	69	50		

TABLE 3 RESULTS FROM ANKOLE REFUGEE PRIMARY SCHOOL

Time of examination	Total examined	Patients with C. diphtheriae ulcers		
	examined	No.	%	
July 1968	12	4	33	
October 1968	14	12	86	
January 1969	28	16	57	
Total	54	32	59	

None of the trunk lesions contained C. diphtheriae. Most patients (80%) had lesions in the form of impetigo. The lesions were usually multiple (in 80% of the patients). Although the small lesions (up to 1 cm in diameter) constituted 42% of negative cases, in C. diphtheriae-positive cases only one

TABLE 4 CLASSIFICATION OF 40 PATIENTS AND THEIR SKIN LESIONS SEEN IN KASANGATI SURVEY

	Total no.	C. diphtheriae			
	of cases	Negative (26)	Positive (14)		
Age (years):					
0–2	7	5	2		
2-4	14	10	4		
515	12	9	3		
>15	7	2	5		
Tribe:					
Baganda	26	19	7		
Others	14	7	7		
Site of lesion:					
Arm	0	0	0		
Leg	13	10	3		
Arm & leg	11	4	7		
Head & neck	9	5	4		
Trunk	7	7	0		
Type of lesion:					
Impetigo	32	22	10		
Ulcer	8	4	4		
Number of lesion:					
Single	8	6	2		
Multiple	32	20	12		
Size of lesion (cm);					
<1	12	11 (42%)	1 (7%)		
1–3	28	15	13		
Duration of lesion (weeks):					
04	22	19 (73%)	3 (21%)		
58	16	5	11		
>8	2	2	0		

lesion was small. All other diphtheria-positive lesions were larger (1-3 cm in diameter). The duration of 73% of diphtheria-negative but of only 21% of the positive lesions was less than 4 weeks. None of the positive lesions lasted less than 2 weeks.

In the Ankole survey 54 children were studied (Table 5). Among 54 pupils, mostly aged 5-15,

TABLE 5 CLASSIFICATION OF 54 PATIENTS AND THEIR SKIN LESIONS SEEN IN ANKOLE SURVEY

	Total no.	C. diphtheriae			
	of cases	Negative (22)	Positive (32)		
Age (years):					
5–15	50	20	30		
>15	4	2	2		
Site of lesion:					
Arm	4	4	0		
Leg	50	18	32		
Number of lesion:					
Single	42	16	26		
Multiple	12	6	6		
Size of lesion (cm):					
<1	40	17	23		
1–3	13	5	8		
>3	1	0	1		
Duration of lesion (weeks):					
0-4	39	14 (64%)	25 ^a (78%)		
58	6	3	3		
>8	9	5	4 ^b		

^a The shortest duration was 3 days in one case.

^b One patient had an ulcer of 9 months' duration.

there were more boys than girls (40:14); only 1 The majority of was not a Ruandan refugee. ulcers, much more frequently single than multiple, were localized on the legs; only a few were on the upper limbs; and there were none on any other part of the body. Those with C. diphtheriae were exclusively leg ulcers. There was no substantial difference in size of diphtheria-positive and -negative ulcers, and in both groups the smaller size was predominant. Diphtheritic ulcers made up just less than two-thirds of the group of acute skin ulcers (duration of up to 4 weeks). The shortest duration of diphtheritic ulcer was 3 days, the longest 9 months.

The results of the typing of the strains of C. *diphtheriae* isolated are shown in Table 6. Among the 74 strains there were 61 *mitis* (82%), 9 atypical and 4 *gravis* strains. In a few children a mixture of two different types of C. *diphtheriae* was found, i.e., *mitis* and *gravis* strains in 2 cases, *mitis* and atypical strains in 3 cases.

TABLE 6 TYPING OF C. DIPHTHERIAE STRAINS ISOLATED FROM SKIN LESIONS

Survey	Total no.typed	Mitis	Gravis	Atypica
Ankole	35	28 (80%)	o	7 (20%)
Karamoja	22	20 (91%)	2 (9%)	0
Kasangati	17	13 (76%)	2 (12%)	2 (12%)
Total	74	61 (82%)	4 (5%)	9 (12%)

Forty-seven strains of *C. diphtheriae* isolated in Ankole and Kasangati were tested for toxin production, and only 2 strains (both *mitis*; 1 from Ankole, 1 from Kasangati) proved virulent; 14 strains showed a very low grade of toxigenicity when tested in guinea-pigs.

TABLE 7						
C. DIPHTHERIAE IN	SKIN	ULCERS	AND	IN	CARRIERS	

Survey	Time of examination	No. throat carriers/ No. examined	No. nasal carriers/ No. examined	No. throat & nasal carriers/ No. examined	Skin carriers
Ankole	October 1968	8/86 (9.3%)		8/86 (9.3%)	12/14 (86%)
Karamoja (Amudat)	March 1969	2/74 (2.7%)	3/74 (4%)	5/74 (6.8%)	8/14 (57%)

In two schools the pupils were examined to see whether any were C. *diphtheriae* carriers; the results are presented in Table 7. In one school, with a high frequency of C. *diphtheriae* skin ulcers, the throat carrier rate was 9.3% while in the other school (with 57% of ulcers containing C. *diphtheriae*) 6.8% were C. *diphtheriae* carriers.

DISCUSSION

Our studies have shown that in Ugandan children and teenagers skin lesions with *C. diphtheriae* are quite common. Depending on the group of children examined, its frequency varied from 32% to 60%. During 1969 in another and more limited study of adults at the Toro Tea Estate (in a north-western district) who were suffering from skin ulcers on the legs, one of us obtained similar results, 20% of the patients being found to have *C. diphtheriae* in their lesions (Bezjak, unpublished data).

In recent years we have also observed adult male patients in Mulago Hospital, Kampala, with *C. diphtheriae* in severe and extensive skin ulcerations of long duration.

Such a frequency of C. diphtheriae in skin lesions is in marked contrast to the rarity of classical diphtheria cases in Uganda (Bwibo, 1969). C. diphtheriae carrier rates of 4%-9% in Ugandan children aged 4-17 years were found at the same time and in the same districts and schools as in the present study (Bezjak & Farsey, 1970b). These carrier rates correspond to those found in other countries where classical diphtheria cases are common and where there are no large-scale immunization programmes.

It is difficult, if not impossible, to compare the observed differences in the frequency of *C. diph-theriae* in the skin lesions of Ugandan children. First, the three groups of children studied differed widely in several respects: in age (from less than 1 year in Kasangati to 18 years in Karamoja); in type of skin lesion; in opportunity for exposure to possible sources of infection (Kasangati children were mostly of the preschool age-group); and in the climatic conditions in which they lived. Secondly, our examinations were made neither simultaneously nor on the same number of occasions. Finally, one group of children (Kasangati) differed from the other two in having been brought to the dispensary because of their skin infections.

The relevant reports by other workers on a larger number of cases from other tropical and subtropical countries in various parts of the world (Table 8) show similar findings for skin lesions infected with *C. diphtheriae*. The secondary role of *C. diphtheriae* was also stressed by Tan Eng Tie (1965), who examined over 300 patients, both children and adults, in prewar and post-war Indonesia and found 27%-32% of skin lesions to harbour *C. diphtheriae*.

Author	No. of <i>C. diphtheriae</i> cases	C. diphtheriae incidence (%)	Patients	Country
Craig (1919)	129	67.5	White soldiers	Middle East
	173	21.5	White soldiers	Pacific Islands
Liebow et al. (1946)	173	21.5	white soldiers	Pacific Islands
Denhoff & Kolodny (1947)	56	68	Soldiers	USA (Texas)
Bacon & Marples (1955)	80	28.7	Samoans	Samoa
Sviridenko (1956)	289	32.5	Whites	USSR (Dagestan)
Markham & Stenhouse (1959)	54	31.8	Maoris	Cook Islands
Tan Eng Tie (1965)	126	32	Mostly Asian	Indonesia
	181	27		
Bennett (1968)	?	about 10	Negro-mestizo	Colombia
Gunatillake & Taylor (1968)	40	40.8	Asians	Ceylon
Belsey et al. (1969)	38	13.6	Whites & Negroes	USA (Louisiana & Alabama

TABLE 8 INCIDENCE OF C. DIPHTHERIAE IN SKIN LESIONS IN VARIOUS COUNTRIES

On the basis of their experience in Colombia and in Alabama and Louisiana, Bennett (1968) and Belsey et al. (1969) expressed a similar opinion. However, when comparing their data with ours and those of other authors, one has to remember that they were obtained by examining all the skin lesions in each patient (Belsey et al.) or by monthly examination of the same group of children throughout a year (Bennett). Therefore, by applying the techniques used by other authors one would get much lower C. diphtheriae rates in skin lesions both in the southern USA and in Colombia. In fact, even with the technique used, i.e., by culturing 1-5 lesions, Bennett obtained monthly rates of 5%-11% of C. diphtheriae skin lesions.

In our study no diphtheria-positive ulcers were found on the trunk, and the upper limbs were only very occasionally involved. The former finding is easily explained by the fact that the body was usually covered by clothing and hence the skin was usually free of injury and much less subject to infection with *C. diphtheriae*. But it is puzzling that in an institution with high *C. diphtheriae* rates (the school at Ankole) not a single diphtheritic ulcer appeared on the arms.

It seems that diphtheritic ulcers proper have a tendency to be single, while the combination of *C. diphtheriae* with multiple lesions is more likely to indicate its being there as a contaminant or secondary invader. On the other hand, the mere presence of *C. diphtheriae* in a single skin ulcer does not necessarily mean that the ulcer is a diphtheritic one, as was shown by our results in the Karamoja survey, where the lesions clinically were tropical ulcers.

There was one observation made in the Kasangati survey that is worth mentioning. In spite of the fact that in the Kasangati area the large majority of the population were Baganda (a Bantu tribe), among *C. diphtheriae*-positive cases there was an equal number of patients from other tribes (see Table 4). This might have been related to factors such as socio-economic status, washing habits, etc., rather than to any genetic factor.

Marked differences were noted between the frequency of skin lesions containing C. diphtheriae, on the one hand, and the nasal and throat carrier rates on the other hand, in the two schools where these rates were studied. In the Ankole refugee school a very high percentage (86%) of children had skin lesions that contained C. diphtheriae, while at the same time only 9.3% of their classmates were throat car-

riers (not a single nasal carrier was found). In Amudat (Karamoja) 57% of the skin lesions had C. *diphtheriae*, while the carrier rate was 6.8% (there was only 2.7% of throat carriers). All this may indicate that the skin lesions offer much better ground for the multiplication of C. *diphtheriae* than the pharyngeal or nasal mucosa, at least in tropical conditions. Similar discrepancies between the low frequency of throat and nasal carriers and the high frequency of C. *diphtheriae* in skin lesions (30%-60%) were also noted in Polynesians by Marples (1965) and by some other authors.

The results of our survey in the Ankole refugee school (Table 3) merit further discussion in view of the widely differing C. diphtheriae rates found on three different occasions. Prior to the October 1968 visit there was an outbreak of streptococcal throat infections in the school, which may have provided a suitable substratum for the establishment of C. diphtheriae carrier status, which showed quite a high rate (9.3%) after the outbreak. These carriers may have in turn served as sources of infection for new cases of skin diphtheria ulcers among their classmates. This way of transmission (from throat to skin) was presumably also responsible for the spread of skin diphtheria in children studied by Sviridenko (1956), because most of them appeared during the period from September to January. The suggested course of events in our school could explain the unusually high percentage (86%) of skin lesions with C. diphtheriae found subsequently in October. A comparison of the patients' names has shown that not a single pupil has had a C. diphtheriaepositive ulcer on more than one occasion, and that none of the C. diphtheriae throat carriers had any skin ulcers during the 7-month period of our studies.

The predominance of the mitis type of C. diphtheriae in skin lesions as reported by us, as well as by all authors who typed the strains they isolated, needs some comment. Although we know that the existence of the prevailing type of C. diphtheriae is subject, at least in a given country, to minor or major changes over a given period of time, this almost exclusive presence of the *mitis* type in skin lesions is very striking. Unfortunately, there is no report in the literature giving the exact respiratory carrier rates (down to the types concerned) in a group of patients harbouring C. diphtheriae in their skin lesions. The gravis type of C. diphtheriae, which has been so important in causing classical diphtheria in a number of countries, was found only comparatively rarely in skin lesions, i.e., in 13%-18%

of cases by Tang Eng Tie (1965), in 3% of patients by Gunatillake & Taylor (1968) and in 6% of cases in the present series.

The toxigenicity of C. diphtheriae strains isolated from skin lesions is surprisingly low, as noted by The number of non-toxigenic various authors. strains was as high as 98.5% (Tan Eng Tie, 1965), while only exceptionally as low as 34% (Belsey et al., 1969). In this connexion the experience of Liebow et al. (1946) was quite remarkable: in white patients with skin lesions only 16% of the isolated C. diphtheriae strains were non-virulent, while at the same time in all indigenous patients from some Pacific Islands only non-virulent C. diphtheriae was isolated. Does this mean that the diphtheria antitoxic immunity is the factor deciding whether the skin is going to be infected with a non-toxigenic strain? Or is it that the duration of skin lesions determines whether or not toxigenic C. diphtheriae could be isolated—the duration being inversely proportional to the frequency of toxigenic strains (Craig, 1919; Liebow et al., 1946)? Our results (96% of non-toxigenic strains) are close to those from Indonesia (Tan Eng Tie, 1965). Bennett's figures show large differences in the monthly rates of non-toxigenic strains isolated from skin lesions; these were between 42% (in May) and 91% (in March), giving a yearly average rate of 64%. That even non-toxigenic C. diphtheriae strains could produce skin ulcers in man under experimental conditions was reported from India by Pasricha & Panja (1940).

There is a pronounced discrepancy between the low percentage of toxigenic strains in skin lesions and the high degree of diphtheria immunity in children and adults in tropical countries. In view of the scarcity of classical diphtheria cases and the absence of active immunization procedures—at least until very recently—the most probable explanation lies in the frequent *C. diphtheriae* skin lesions. Unpublished findings by Farsey of over 90% of Schick-negative children in non-immunized school pupils (aged 10–15 years) in the Kasangati Health Centre area during 1969-70, and by Arya of more than 80% of Schick-negative Makerere medical students (African) during 1966-69, give further support to our theories. But here again there is another question: how does diphtheria immunity develop so quickly, and quite early in childhood, if most skin strains of C. diphtheriae do not produce toxin? That even persons with skin lesions containing non-toxigenic C. diphtheriae could develop late diphtheritic paralyses has been reported by some authors (Denhoff & Kolodny, 1947). It has been stated that "avirulent" skin strains may produce minute amounts of toxin, insufficient to be demonstrable by our present laboratory techniques (Marples & Bacon, 1956). The only other satisfactory hypothesis that occurs to us is that a single skin lesion may contain either a mixture of toxigenic and non-toxigenic organisms, or that at least one of the existing multiple lesions may contain toxigenic C. diphtheriae. In fact it seems that both of these assumptions are correct, as was shown by Bennett's experience in 1969 (personal communication). Testing 1-5 C. diphtheriae colonies from each patient's sample, he found a mixture of both toxigenic and non-toxigenic strains in 5% of cases; on the other hand, among patients with multiple skin lesions, 17% had toxigenic C. diphtheriae in some lesions and non-toxigenic C. diphtheriae in others.

There is still another possible explanation, which could apply when toxigenic strains circulate in a closed community, as demonstrated again by Bennett (personal communication) in Buenaventura, Colombia, during 1969: both the replacement of a non-toxigenic by a toxigenic strain in skin lesions and of a toxigenic by a non-toxigenic strain were noted. Most probably this was not due to the loss or, still less likely, to acquisition of the toxigenic property of the organism present in the lesion, as also concluded by Kostyukova & Favorova (1968), who used quite sophisticated techniques when studying C. diphtheriae in patients and carriers during an epidemic of classical diphtheria in the USSR.

RÉSUMÉ

CORYNEBACTERIUM DIPHTHERIAE DANS LES LÉSIONS CUTANÉES CHEZ DES ENFANTS OUGANDAIS

Des prélèvements effectués in situ chez 139 écoliers ougandais atteints de lésions cutanées infectées (impétigo, ulcères tropicaux) ont montré chez 69 d'entre eux (50%) la présence de Corynebacterium diphtheriae. Dans une école, l'enquête poursuivie pendant plusieurs mois a permis d'isoler le bacille diphtérique chez respectivement 33% (juillet 1968), 86% (octobre 1968) et 57% (janvier 1969) des jeunes malades. Ailleurs, 60% des écoliers atteints d'ulcères tropicaux étaient infectés localement par le bacille. Le taux d'infection le plus bas (32%) a été enregistré dans un centre de santé. Dans deux groupes d'enfants de ces mêmes établissements, *C. diphtheriae* n'a été isolé au niveau des voies respiratoires que chez 9,3% et 6,8% d'entre eux.

L'étude clinique a révélé certaines particularités des lésions infectées par *C. diphtheriae*: elles sont généralement uniques, de plus grande taille et d'évolution plus lente.

Lors du typage des souches isolées, 82% d'entre elles

ont été reconnues comme appartenant au type *mitis* et 5% au type *gravis*; 12% des souches étaient atypiques. Sur 47 souches inoculées au cobaye, 2 seulement (4%) ont fait preuve de virulence.

La rareté de la diphtérie, sous sa forme classique, en Ouganda et l'absence de tout programme de vaccination antidiphtérique dans ce pays amènent les auteurs à attribuer la protection, appréciable et répandue, de la population à un processus d'immunisation naturelle résultant de la présence fréquente de C. diphtheriae dans les lésions cutanées.

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