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ORIGINAL ARTICLE

M. Große Perdekamp · B. P. Kneubuehl · A. Serr · B. Vennemann · S. Pollak

Gunshot-related transport of micro-organisms from the skin of the entrance region into the bullet path

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Abstract The skin defect of a gunshot entrance wound is caused by the retrograde and anterograde displacement of skin particles. In the present study, we investigated whether gunshots to bacterially contaminated skin are associated with the transport of micro-organisms into the bullet path. The shots were fired into composite models of pig skin and gelatin blocks. The outer surface of the skin was covered with a thin layer of a defined bacterial suspension [green fluorescent protein-labelled *Escherichia coli* in the preliminary test and *Staphylococcus epidermidis*, DSM 1798, in the main test series]. After the bacterially contaminated fluid had dried, test shots were fired from a distance of 5 and 10 m using calibre .38 Special cartridges with different bullet types (round nose, truncated cone, hollow point and flat nose). Subsequent bacteriological analyses showed that all the bullet tracks in the gelatin serving as tissue simulant contained displaced micro-organisms from the skin surface. The results are presented and discussed with reference to the transport of skin particles into the depth of the wound track.

Keywords Gunshot wound · Skin flora · Composite model · Bacterial contamination

M. Große Perdekamp (✉) · B. Vennemann · S. Pollak
Institute of Legal Medicine,
University Hospital Freiburg,
Albertstraβe 9,
79104 Freiburg, Germany
e-mail: markus.perdekamp@uniklinik-freiburg.de
Tel.: +49-761-2036875
Fax: +49-761-2036858

B. P. Kneubuehl
Armasuisse, Feuerwerkerstrasse 39,
3602 Thun, Switzerland

A. Serr
Institute of Medical Microbiology and Hygiene,
University Hospital Freiburg,
Hermann-Herder-Straβe 11,
79104 Freiburg, Germany

Introduction

In gunshot entrance wounds, a central roundish hole (due to a local tissue defect) is regarded as a relatively characteristic feature often permitting differentiation from exit wounds. As former studies have shown, the defect is caused by the retrograde and anterograde displacement of macroscopically discernible, but also of microscopically small skin particles [1]. It could be demonstrated that skin particles are carried along the whole length of the permanent bullet track and also into the peripheral parts of the temporary cavitation. Under normal conditions the skin surface of healthy individuals is populated by various micro-organisms (so-called resident microbial flora) so that the question arises whether bacteria of the entrance site are carried into the depth of the wound track, which—if the victim survives—may constitute a potential source of infection. Therefore, we performed experiments using composite models consisting of pig skin contaminated with bacteria and gelatin blocks to investigate (1) whether a transport of micro-organisms can be demonstrated (see preliminary test) and (2) whether there is a topographical relation between the final position of the displaced skin particles and the bacteria found in the wound track (see main test series).

Materials and methods

Preliminary test with green fluorescent protein (GFPuv)-labelled *Escherichia coli*

A piece of skin measuring 5×5 cm from the belly region of a slaughtered pig was disinfected then air-dried and covered with a suspension of recombinant *Escherichia coli* DH5α K12, which constitutively expresses green fluorescent protein (GFPuv) after transformation with the respective plasmid. After air-drying the skin specimen again, it was attached to the front side of a rectangular gelatin block (as shown in a previous paper [1]).

For primary disinfection the piece of skin was submerged in 70% ethanol (Promanum N, Braun Melsungen, Melsungen, Germany) for 30 min then air-dried and stored in a sterile container. Directly before firing the test shot, 500 µl of a bacterial suspension (GFP-labelled *E. coli*, 10^8 CFU/ml) was applied to the skin surface with a sterile cotton swab.

The gelatin block (26×12×12 cm in size) was made from gelatin powder (250 Bloom, type A, grain 20/60) from Naumann–Gelatine and Leim (Memmingen, Germany), which had been pretested for sterility. Preparation of the simulant and the further processing and storage of the block were carried out under sterile conditions according to current guidelines [2].

The test shot was fired from a revolver (Smith & Wesson six-shot double-action revolver, model 14-1, calibre .38 Special) from a distance of 5 m using a Hirtenberger round nose lead bullet cartridge (Hirtenberg, Austria). The inside of the barrel, the cartridge chamber and the cartridge surface were disinfected before firing the shot.

After the shot the gelatin block was laminated in 1-cm-thick layers under sterile conditions, and the section of the bullet track was punched out from each layer. Thus, the gelatin punches obtained had a volume of approximately 500 µl. They were liquefied at 37°C then spread on Luria–Bertani (LB) agar with 100 mg/l ampicillin and incubated at 36±1°C for 48 h. After 24 and 48 h the culture media were examined for bacterial growth, and smears of bacterial colonies were investigated by fluorescence microscopy.

Main test series with *Staphylococcus epidermidis*

After a maximum storage time of 2 days at 4°C, nine pieces of pig skin, each measuring 5×5 cm, were incubated with haematoxylin to stain them blue. The pig skin was stained by submerging it in a haematoxylin bath for 60 min (Mayer's haemalum, Merck, Darmstadt, Germany). Subsequently, the dyed pieces of skin were dried with absorbent paper and stored in airtight containers at 4°C until the test shots were performed.

Before firing the shots (nine in all, see Table 1), the pieces of skin were disinfected as described, air-dried and stored separately in sterile containers. Directly before the test shots the bacterial suspension (*Staphylococcus epidermidis*, DSM 1798, 10^8 CFU/ml) was applied to the skin surface (Fig. 1a), and the pieces of skin were air-dried and attached to the front sides of the gelatin blocks.

For firing the shots (Fig. 1b) a mount, calibre .38 Special and different cartridges, whose surface had been disinfected with alcohol, were used (Table 1). After lamination (Fig. 1c) of the gelatin blocks, each layer was examined for its content of blue-coloured pig skin particles, and the number and size in the macroscopically visible range was evaluated semi-quantitatively (scores 1, 2, 3 indicate low, medium and high density respectively; Fig. 2).

Then, the section containing the bullet track was punched out from each layer and processed using Columbia blood agar as culture medium (Fig. 1d). Specimens collected away from the bullet track in randomly selected gelatin layers were examined as negative controls. Moreover, one shot was fired to a piece of skin that had been disinfected, but not contaminated with bacteria.

After an incubation time of 24 h the bacterial colonies were counted, and representative colonies from each block were subcultured onto Columbia blood agar and subsequently classified by means of standard methods (Gram staining and catalase test). Species identification was based on ID 32 Staph (bioMérieux, Marcy-l'Etoile, France). After 48 h Columbia blood agar plates were inspected again for bacterial growth.

Results

Preliminary test

The projectile passed through the whole length of the composite model of pig skin and gelatin, thus producing a 26-cm-long bullet track. After laminating and punching out the gelatin around the permanent bullet track, 26 smear cultures were incubated for 24–48 h and examined for GFP-labelled *E. coli* colonies. Growth of GFP-labelled bacterial colonies in the bullet track was demonstrated by means of fluorescence microscopy. No other microorganisms were found.

Main test series

Distribution of skin particles along the bullet track

All shots fired in the test series perforated the composite models completely. In all the 26-cm-long bullet tracks macroscopically discernible skin particles were found (Fig. 2). With round nose lead bullets, skin particle density was high at first, then declined and increased again towards

Table 1 Ammunition data of the cartridges used for test shots

Number of shots	Bullet shape	Abbreviation	Bullet type	Bullet mass (g)	Bullet velocity (m/s)	Bullet energy (J)	Manufacturer
6	Round nose	rn (1–6)	Lead	10.2	226–249	260–316	Winchester
1	Truncated cone	tc	Semi-jacketed	7.45	278	288	Winchester
1	Hollow point	hp	Semi-jacketed	8.1	314	399	Remington
1	Flat nose	fn	Lead	9.6	221	234	Remington

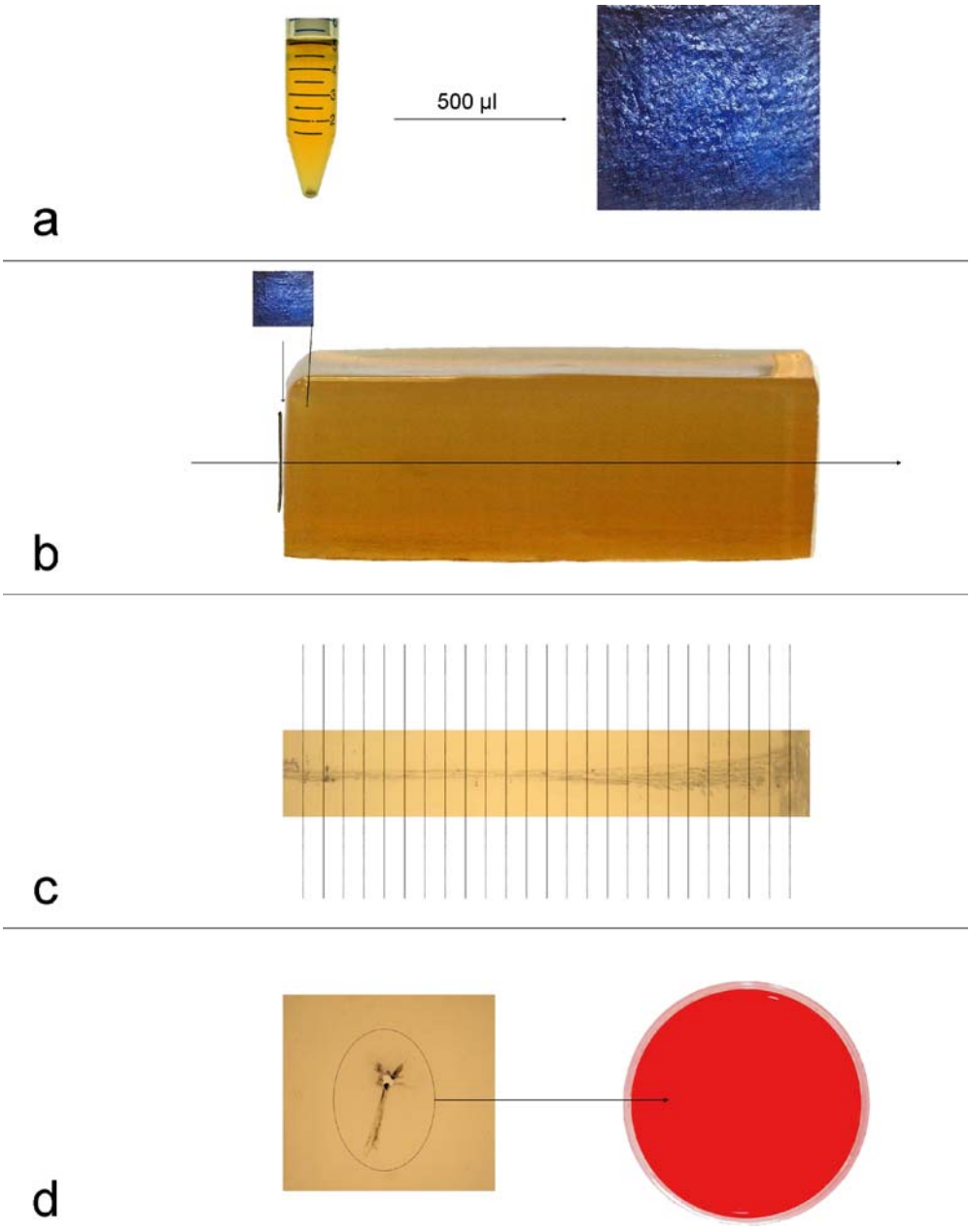


Fig. 1 Schematic illustration of the experimental studies. **a** Disinfected blue-coloured skin from a pig is wetted with a defined bacterial suspension. **b** Shots are fired into the composite model of skin and gelatin from the skin side. **c** After firing the shots, the gelatin blocks are laminated in 1-cm-thick layers. **d** The sections with the bullet track are evaluated semi-quantitatively with regard to skin particle density, then punched out, liquefied and spread on culture media.

the end of the bullet track. With the hollow point bullet, particle density decreased continuously along the bullet path. The track produced by the flat nose bullet was characterized by a relatively even particle distribution, and in shots with the truncated cone projectile, particle density in the bullet track decreased markedly after 10 cm.

Distribution of bacteria along the bullet track

After an incubation time of 24 h most of the punched-out bullet track sections of each gelatin block, except the negative controls, showed growth of *S. epidermidis* colonies. The number of bacterial colonies found varied considerably from block to block even if the same bullet type was used (Fig. 2).

The morphologically uniform pattern of white colonies was consistent with the typical appearance of *S. epidermidis*. Representative colonies from each block were identified as *S. epidermidis* with an identical biochemical profile (as the strain *S. epidermidis* DSM 1789 used for contamination of the skin). In addition minor contamination with Gram-negative rods was detected in seven of the nine examined bullet tracks, which could be morphologically differentiated from the skin bacteria applied for test purposes. At the second inspection after 48 h there was only a moderate increase in contamination (obviously, the growth of these non-fermenting environmental contaminants was slow at 36°C).

An examination of the control block, in which the shot was fired to disinfected skin without previous application of bacteria, only showed growth of five bacterial colonies

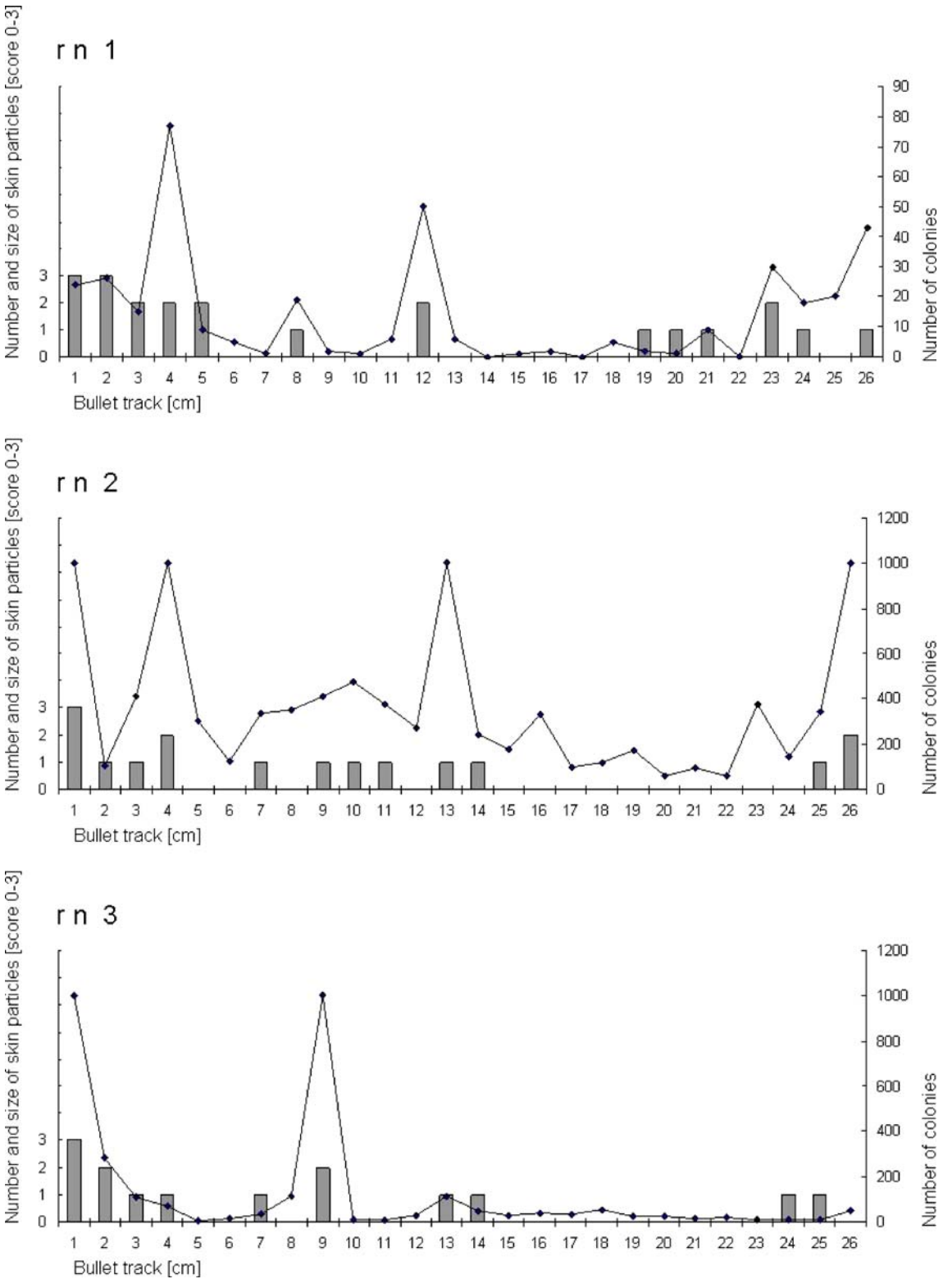


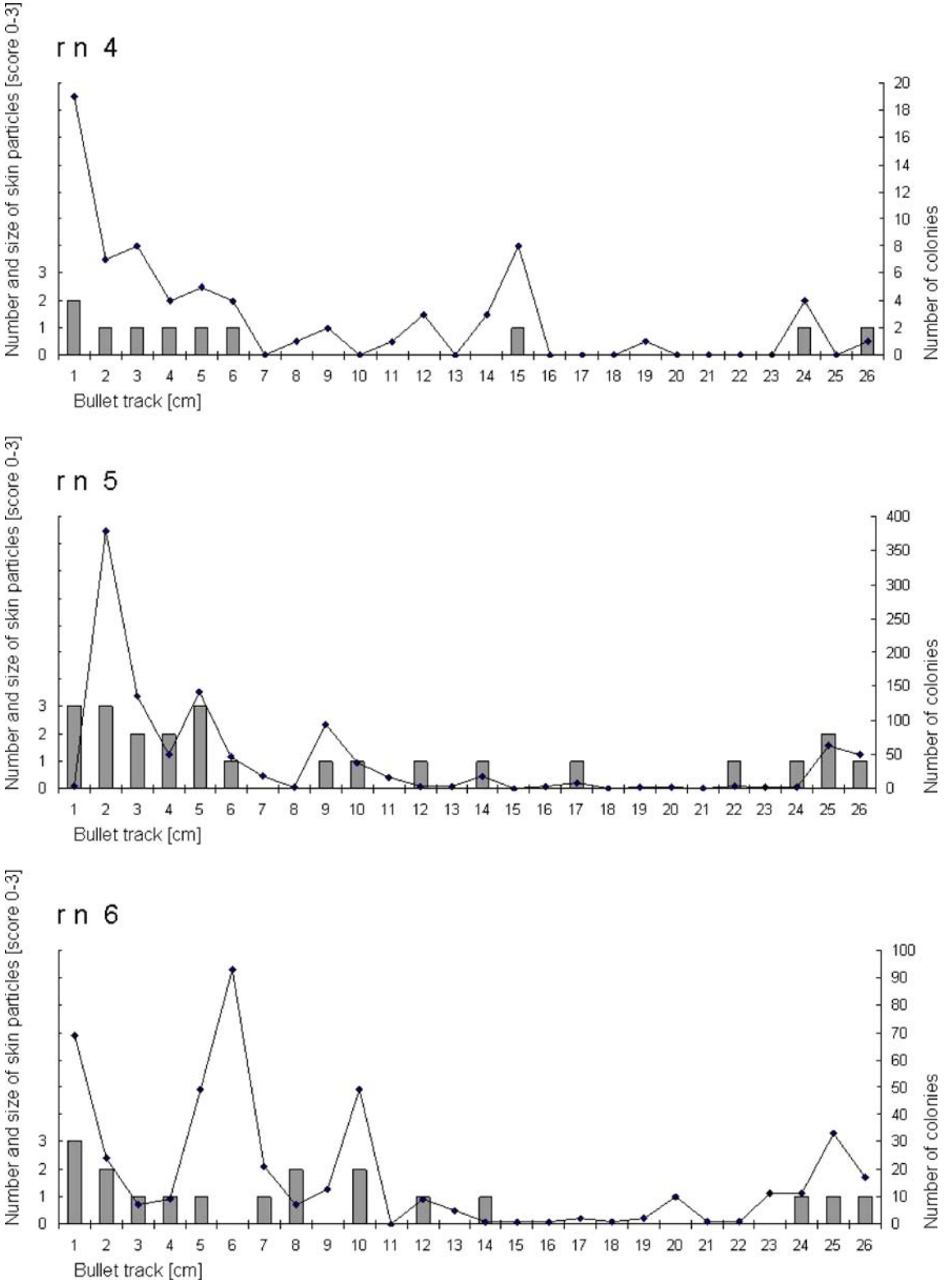
Fig. 2 Comparative illustration of skin particle distribution in the bullet tracks [score 0–3 (■)] and number of bacterial colonies (◆)

in layer 4 (from the front) of the bullet track. This contamination was identified as *Staphylococcus saprophyticus* by means of ID 32 Staph. None of the gelatin samples obtained from regions away from the bullet paths showed any bacterial growth.

Discussion

In surviving victims, local infection of gunshot injuries is a frequently seen complication [e.g. 3, 4]. This problem is of particular significance in times of war [e.g. 5–10]. Clinical

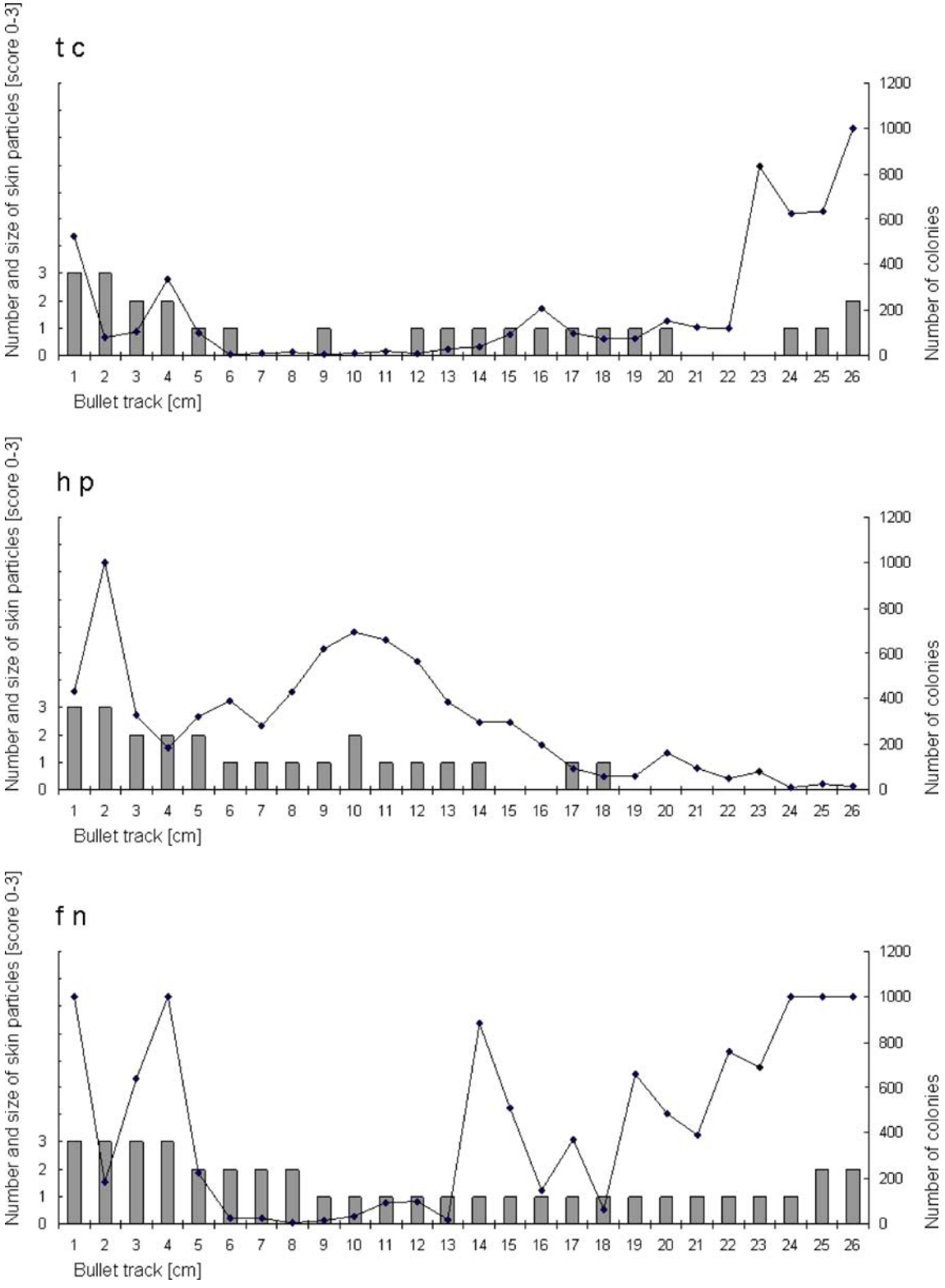
Fig. 2 (continued)



studies usually focus on the question of an optimal prophylaxis/therapy to minimize the risk of infection [e.g. 4, 11, 12], but the mechanism of primary bacterial contamination has rarely been investigated in scientific studies [13, 14].

The hypothesis of "autosterilization" of bullets [15] is regarded as obsolete today. It is known that both bacterially contaminated projectiles [16–18] and bacteria from the clothing and the skin [13, 14] can penetrate into the depth of the wound track. That micro-organisms from the skin

Fig. 2 (continued)



surface are actually carried into the bullet track was confirmed by our preliminary test with GFP-labelled bacteria. The micro-organisms detected along the permanent bullet track could be clearly identified as those bacteria previously applied to the skin of the gunshot entrance region. This proves that an infection of a gunshot injury can be caused by bacteria localized on the skin at the gunshot entrance site. In the preliminary test, negative controls were not considered necessary because fluorescence-labelled bacteria do not occur in the natural

environment, so contamination with this kind of bacterium is not possible.

In a second step, we investigated whether there is a topographic relationship between the skin particles lodged in the bullet track and the bacteria which had been applied to the skin surface prior to the shot. For the shots of the main test series, *S. epidermidis* was spread on the skin to be fired at, as these bacteria are commensal inhabitants of the skin. The negative controls and species identification of representative colonies in each block by random typing of the colonies confirmed that the bacteria found along the bullet path originated from the skin surface.

There are several possible explanations for the minor contamination with other micro-organisms: one possibility is that the gelatin powder already contained bacteria; contamination may also have occurred during one of the numerous procedures. Likewise, micro-organisms may have remained intact in the depth of hair follicles despite the disinfection of the pig skin. Finally, bacterially contaminated gunpowder cannot be ruled out entirely [18].

The distribution of skin particles along the bullet track, which was evaluated semi-quantitatively (score 1–3), was consistent with the results of previous investigations [1]. As each bacterial colony on the investigated culture media is the result of a single displaced skin bacterium, the number of bacteria carried along could be indirectly quantified by counting the bacterial colonies. The large fluctuations between the total colony counts in the different gelatin blocks are most likely due to the fact that the concentration of bacteria on the skin surface was not uniform after drying of the bacterial suspension.

A comparison between the local intensity of skin particle deposition and the corresponding number of skin bacteria colonies revealed certain relations. For example, the remarkable increase in skin particle density towards the end of the bullet track observed with round nose projectiles was associated with an increased number of bacterial colonies in five of the six test shots. Parallel increases and decreases of these two parameters were also seen with the other bullet head types. Taking into account that some tissue particles transported along the bullet track do not have all skin layers, including the epidermis [1], a closer relation was hardly probable. Nor is it surprising that bacteria may occur even in those sections in which no skin particles can be detected macroscopically; in fact, previous investigations have shown that, at least microscopically, small skin particles are present in all sections of the bullet track [1].

The relation between the skin particle distribution and the bacterial colony count along the bullet track suggests that the skin particles of the entrance site may serve as a transport vehicle of the adherent micro-organisms. Apart from a direct displacement by the bullet itself, the suction effect of the temporary cavitation could also be of major importance especially near the entrance and exit region. That the suction effect is actually powerful was demonstrated by Tian et al. [13]: for test shots to the legs of dogs, a bacterially contaminated cloth (containing *Bacterium prodigiosum*) was placed on the side where the bullet exited. Bacteria were detected from the exit wound to the middle of the wound track, for which a suction mechanism is the only plausible explanation.

The cartridges used were chosen in accordance with the design of the preceding study [1] to ensure comparable investigation conditions and results. For practical reasons the tests had to be confined to a small number of cartridges so that, for the time being, a cautious interpretation of the results is advisable. Further studies with a larger number of test shots and additional types of cartridges, including those with full metal jacketed (FMJ) bullets will be performed in the near future.

The findings described in the present paper once more demonstrate the importance of an interdisciplinary approach to forensic wound ballistics, as was also recently shown by other medico-legal authors [19–28].

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