# Safety, tolerability and efficacy of intradermal rabies immunization with DebioJect™

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## ARTICLE INFO

## ABSTRACT

In a single-center study, 66 healthy volunteers aged between 18 and 50 years were randomized to be immunized against rabies with three different injection routes: intradermal with DebioJect™ (IDJ), standard intradermal with classical needle (IDS), also called Mantoux method, and intramuscular with classical needle (IM). "Vaccin rabique Pasteur®" and saline solution (NaCl 0.9%) were administered at D0, D7 and D28. Antigen doses for both intradermal routes were 1/5 of the dose for IM. Tolerability, safety and induced immunogenicity of IDJ were compared to IDS and IM routes. Pain was evaluated at needle insertion and at product injection for all vaccination visits. Solicited Adverse Event (SolAE) and local reactogenicity symptoms including pain, redness and pruritus were recorded daily following each vaccination visit. Adverse events (AE) were recorded over the whole duration of the study. Humoral immune response was measured by assessing the rabies virus neutralizing antibody (VNA) titers using Rapid Fluorescent Focus Inhibition Test (RFFIT). Results demonstrated that the DebioJect™ is a safe, reliable and efficient device. Significant decreases of pain at needle insertion and at vaccine injection were reported with IDJ compared to IDS and IM. All local reactogenicity symptoms (pain, redness and pruritus) after injection with either vaccine or saline solution, were similar for IDJ and IDS, except that IDJ injection induced more redness 30 min after saline solution.

No systemic SolAE was deemed related to DebioJect™ and classical needles. No AE was deemed related to DebioJect™. No Serious Adverse Event (SAE) was reported during the study.

At the end of the study all participants were considered immunized against rabies and no significant difference in humoral response was observed between the 3 studied routes.

## 1. Introduction

Most vaccinations today consist in administering a high amount of antigens via the intramuscular (IM) route. Because of the cost of these antigens and sometimes their shortage in availability, their use is limited in many developing countries. Intradermal (ID) administration of several vaccines has been shown to require lower amounts of antigen than IM vaccination, therefore providing a significant economic advantage [1–3]. Four licensed vaccines are currently delivered ID: smallpox (vaccinia), BCG, influenza (INTANZA®/IDflu®, Sanofi Pasteur) and rabies. In particular, in the case of rabies and influenza, several studies have shown that reduced doses (typically 10% or 20% of the standard amount of antigen) delivered ID could induce immune responses similar to those seen with the standard dose through IM route [1,4–6]. For these reasons, ID was confirmed to be a promising method for vaccination [7].

Skin is considered as a desirable vaccination target since dermis and epidermis layers are rich sources of antigen-presenting cells (i.e. Langerhans cells, dermal dendritic cells and dermal macrophages) which are known to participate in vaccine induced immune responses [8,9]. In addition, the dense network of blood capillaries and lymphatic vessels present within the dermis greatly facilitates the trafficking of leukocytes and dendritic cells from skin to the secondary lymphoid organs. However, the Mantoux method is difficult to execute properly in order to ensure a full delivery into the dermis. The technique requires specific training and regular practice of healthcare workers [1,10–12]. Therefore new delivery devices simplifying intradermal injections and rendering them less

user-dependent are needed in order to target the skin dermis with its specific immune properties more effectively.

DebioJect™ has been designed for this very purpose. The device is based on a hollow microneedle, whose length is limited to 750 μm to avoid most pain receptors. The microneedle is made of very strong monocrystalline silicon covered with biocompatible silicon dioxide. It has an extremely sharp tip and a lateral delivery aperture located at 500 μm from the base to be minimally invasive and to ensure drug administration into the dermis without risking blocking the injection channel (Fig. 1).

DebioJect™ is designed for easy use by medical staff after a very short training. It is CE marked and can be connected to any standard syringes. An inserter is used to ensure the full penetration of the microneedle into the skin in every circumstance.

ID rabies vaccination is promoted by the World Health Organization (WHO) [13], and has been established for post-exposure prophylaxis in India, the Philippines, Sri Lanka and Thailand [14–18]. Rabies is often considered as a good vaccine candidate for the evaluation of new intradermal device delivery systems [1].

The worldwide burden of human rabies is at an estimated number of 55,000 deaths per year, occurring predominantly in Asia and Africa along with canine rabies [13,19–21]. As no effective treatment is available, vaccination is essential to prevent rabies at both pre- and post-infection stages [16].

Efficient purified rabies vaccines produced in cell-cultures or embryonated eggs were developed more than four decades ago [22]. The "Vaccin rabique Pasteur®" manufactured by Sanofi Pasteur, a purified rabies vaccine cultured on Vero cells, is WHO-approved for pre- and post-exposure prophylaxis by ID and IM routes. WHO recommends a 5-fold antigen dose reduction for the intradermal route compared to the IM route, which is expected to induce a rabies virus neutralizing antibody (VNA) response $\geqslant 0.5$ IU per mL (International Unit/milliliter), considered as sufficient for protection against rabies [4,22–24].

The objectives of this study were to evaluate DebioJect™ (IDJ) safety, tolerability and induced immunogenicity compared to standard intradermal/Mantoux (IDS) and IM route in the frame of rabies vaccination with "Vaccin rabique Pasteur®".

# 2. Materials and methods

## 2.1. Ethics statement

The study was conducted according to Good Clinical Practice, the Declaration of Helsinki, Directive 90/385/CEE and 93/42/CEE, International Standa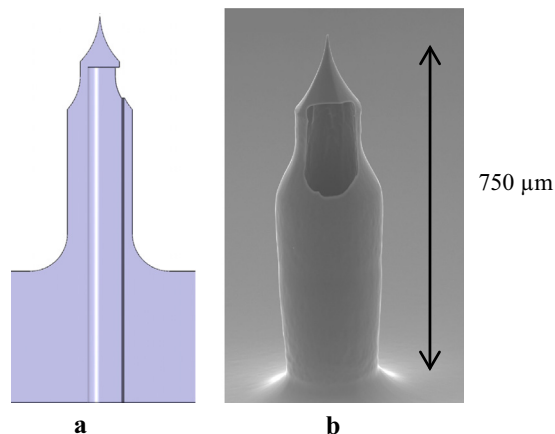rd ISO 14155:2011. The protocol was first approved by the local Institutional Review Board (*Commission cantonale (VD) d'éthique de la recherche sur l'être humain*, reference: 178/12) on July 13th, 2012, and then by Swissmedic, the Swiss Agency for Therapeutic Products (reference: 2012-MD-0030) on June 3, 2013. EUDAMED Identifier: CIV-12-12-009346. ClinicalTrials.gov Identifier: NCT02538185.

## 2.2. Study design

The study was planned as a single-center, Phase I, first-in-human pilot study to assess the safety and tolerability of the DebioJect™ device, and the immunogenicity of the rabies vaccine "Vaccin rabique Pasteur®" delivered with the DebioJect™ device by intradermal route. In addition to vaccine, saline solution (NaCl 0.9%; B. Braun) was injected in order to complement safety and tolerability data. Saline solution was not intended to be used as a placebo in the immunogenicity evaluation of the "Vaccin rabique Pasteur®".

The study was conducted at the Vaccine and Immunotherapy Center (VIC), which is a specialized unit of the Service of Immunology and Allergy of the Centre Hospitalier Universitaire Vaudois (CHUV) in Lausanne, Switzerland, from August 2013 to September 2014. Both volunteers and investigators were blinded regarding the injected substance although they both knew the device used to perform each injection.

## 2.3. Assessment of IDJ injection completeness

One of the main issues of ID injections based on microneedle is incomplete injection due to leakage [25]. Injection with IDJ was considered to be complete if leakage (non-injected fluid residue present on the skin) represented less than 10% of the volume of the product to be injected. A procedure was implemented to measure the amount of non-injected liquid for each IDJ injection. The procedure consisted of three steps: (1) a blotting paper hermetically sealed in an Eppendorf tube was weighed. (2) The non-injected fluid residue present on the skin was immediately absorbed after the injection with the blotting paper. The blotting paper was replaced in the Eppendorf tube which was then hermetically sealed. (3) Finally the Eppendorf tube containing the wetted blotting paper was weighed again with the amount of non-injected fluid residue corresponding to the weight difference between both measurements.

## 2.4. Investigational and comparator devices

The investigational device was DebioJect™ developed by Debiotech. The comparator device used for IDS was a 25G needle mounted on a standard syringe. The comparator device used for IM injection was a 22G needle mounted on a standard syringe. The operators involved in the study were selected upon their ability and experience to perform successful Mantoux injection. They were also trained to use DebioJect™.

## 2.5. Study population

Subjects were recruited in the study only if they were aged between 18 and 50 years and in good general health, confirmed by medical history, physical examination and screening laboratory tests. Female subjects were required to avoid pregnancy through the duration of the study.

The following criteria caused the exclusion of the study group: an oral body temperature $\geqslant 37.5$ °C; any history or evidence of rabies vaccination or rabies contact, autoimmune disease, any possible immunodeficiency state, including HIV-1 infection, and of chronic hepatitis; any injection of immunoglobulin or blood



**Fig. 1.** Schematic cross section (a) and SEM picture (b) of DebioJect™'s hollow microneedle with lateral aperture.

products within 90 days prior to study visit 2, any investigational drug therapy or investigational vaccine within 180 days prior to study, any licensed vaccine within 45 days prior to study visit 2, Chloroquin and/or Proguanil treatment within 420 days prior to study [26]; any use of immunosuppressive drugs or anticoagulants; any Body Mass Index (BMI) ⩽ 18 or ⩾ 33; and any immediate need of rabies immunization.

Ninety-three candidates provided written informed consent at screening visit. 27 were considered as not eligible, most of which not meeting the medical inclusion criteria. 66 participants were enrolled and randomly assigned to one of 3 groups of 22 participants (group A, B, or C).

The CHUV pharmacist generated a block balanced randomization sequence. According to group allocation, participants were injected with rabies vaccine and saline solution as described in Table 1. The sequence for each participant consisted of the following five visits: one screening visit, three vaccination visits at time points D0, D7, D28, and one follow-up visit at D56. Study duration per volunteer was 56 days from the first vaccination to the last follow-up visit. Table 2 summarizes demographic characteristics of the subjects enrolled in the study. Due to protocol deviation, 1 subject from group B and 1 from group C were excluded from analyses.

As age and BMI of the enrolled population were distributed closely to the corresponding mean values in each study group, these parameters were not considered as covariates for statistical analyses of safety and immunogenicity results.

### 2.6. Study product administration

The doses of vaccine injected were 0.1 ml for both ID routes and 0.5 ml for IM route. IDJ injections were performed in the right forearm and IDS in the left one. IDJ and IDS injections were performed at three different locations corresponding to defined time points: 5 cm (D0), 8 cm (D7) and 11 cm (D28) from the "fossa cubitalis". IM injections were performed in the non-dominant deltoid muscle

at three different locations corresponding to defined time points: 5 cm (D0), 8 cm (D7) and 11 cm (D28) from the acromion.

### 2.7. Study vaccine

The vaccine used in this study was the "Vaccin rabique Pasteur®" manufactured by Sanofi Pasteur (Lot number J1336, expiration date May 2015), a WISTAR PM/WI 38-1503-3M strain rabies vaccine, cultured on Vero simian cell cultures, then purified, inactivated by β-propiolacton and lyophilized. "Vaccin rabique Pasteur®" was reconstituted in the appropriate solvent before being administrated.

### 2.8. Assessment of safety and tolerability

Pain intensity at needle insertion and during vaccine and saline solution injections was based on volunteers' self-evaluation using a Visual Analog Scale [27] (scores ranged from 0 to 10). At each site, when incomplete injection using IDJ occurred, all pain scores (i.e. also from the IDS and IM injections) were discarded.

Adverse Events (AE) were categorized as Solicited AE (SolAE) and Unsolicited AE. SolAE were predefined in the subject's Case Report Form (CRF). SolAE were recorded within the reactogenicity period (30 min after the injection, the evening following the injection and daily thereafter for 3 days and until the AE was resolved). Local SolAE are Pain, Redness and Pruritus at injection site. Pain subsequent to injection was evaluated during reactogenicity period at each injection site using the following scores: 0 for "absence of pain", 1 for "painful on touch", 2 for "spontaneously painful". Redness was assessed using the following score: 0 for "absence of redness", 1, 2 and 3 considering redness diameters ⩾ 3 mm, ⩾ 20 mm and ⩾ 50 mm, respectively. Pruritus was assessed using the following score: 0 for "absence of pruritus", 1 for "mild intensity", 2 for "moderate intensity", and 3 for "severe intensity".

Systemic SolAE were listed as follows: headache, malaise, fever (⩾ 38 °C), chills, asthenia, nausea, arthralgia, myalgia, dizziness and gastrointestinal disorders. All AE were evaluated by the investigator and recorded in the subject's CRF.

### 2.9. Assessment of immunogenicity

The immunogenicity was assessed by measuring the humoral response. Rabies VNA titers were analyzed in serum samples obtained at each visit by Rabies Neutralization Test using the Rapid Fluorescent Focus Inhibition Test (RFFIT; [28,29]). An antibody titer of 0.5 IU/mL was considered as the threshold for protective immunization of the subjects [22].

### 2.10. Statistical analyses

All safety and immunogenicity data analyses were performed at the VIC and at the Swiss Rabies Center of the Institute of Virology and Immunology. GraphPad Prism 6, R-software (R Development

**Table 1**
Groups allocation. The 66 subjects were allocated in three groups. **Group A**: subjects received vaccine through IDS route; **Group B**: subjects received vaccine through IDJ route; **Group C**: subjects received vaccine through IM route.

| Groups | Number of volunteers | Route and volume of administration at three time points: D0, D7, D28 |
|--------|----------------------|----------------------------------------------------------------------|
| A | 22 | Right arm : IDJ with saline solution (0.1 mL) <br> Left arm : **IDS with vaccine** (0.1 mL) <br> Non-dominant deltoid : IM with saline solution (0.5 mL) |
| B | 22 | Right arm : **IDJ with vaccine** (0.1 mL) <br> Left arm : IDS with saline solution (0.1 mL) <br> Non-dominant deltoid : IM with saline solution (0.5 mL) |
| C | 22 | Right arm : IDJ with saline solution (0.1 mL) <br> Left arm : IDS with saline solution (0.1 mL) <br> Non-dominant deltoid : **IM with vaccine** (0.5 mL) |

**Table 2**
Demography. Subjects received vaccine trough standard IDS (Group A), IDJ (Group B), and IM (Group C) routes.

| | | Group A <br> **22** | Group B <br> **21** | Group C <br> **21** | Overall <br> **64** |
|---|---|---|---|---|---|
| Number of subjects | | | | | |
| Female number (%) | | 9 (40.9) | 10 (47.6) | 12 (57.1) | 31 (48) |
| Male number (%) | | 13 (59.1) | 11 (52.4) | 9 (42.9) | 33 (52) |
| Age [years] | Min/Max | 18.6/43.3 | 20.4/43.6 | 19.6/36.9 | 18.6/43.6 |
| | Mean ± SD | 25.4 ± 6.4 | 25.8 ± 6.3 | 23.9 ± 4.0 | 25.1 ± 5.6 |
| BMI [kg m$^{-2}$] | Min/Max | 18.34/31.64 | 19.03/28.41 | 18.14/30.72 | 18.14/31.64 |
| | Mean ± SD | 22.8 ± 3.4 | 22.6 ± 2.6 | 22.2 ± 3.1 | 22.6 ± 3.0 |

The bold values represents the number of subjects per group (e.g. 22 subjects in group A). In the last column, it represents the number of subjects for the three groups.

Core Team, 2010) and NCSS 2007 software were used for all the statistical analyses.

Pain intensities at needle insertion and at product injection site were compared between injection devices using the Mann Whitney test. Pain, redness and pruritus at injection site were compared between groups following vaccination for each time point of the reactogenicity period using a 2-way analysis of variance (ANOVA) combined with Tukey's multiple comparison tests. Comparison of RFFIT results between injection devices at the last study visit was performed using a Kruskal-Wallis test combined with a Dunn's multiple comparison test. Rabies VNA titers were compared between study groups at each study visit using an ANOVA (repeated measures) test combined with a Bonferroni Multiple Comparison Test.

### 2.11. Data exclusion

Due to protocol deviation, 1 subject from group B and 1 from group C were excluded from analyses reducing the number of subjects from 66 to 64 and the total number of injections included in the analyses from 198 to 192 for each injection route (IDJ, IDS, IM).

All IDS and IM injections were successfully performed. Regarding IDJ, 165 injections were considered as complete while 27 injections were considered as incomplete. Until the 75th injection, the rate of IDJ complete injections was low at 67% (50 complete injections out of 75). After an additional training of the operators, the rate of IDJ complete injection reached 98% (115 complete injections out of 117). The two failures were due to an operator error during the device preparation.

## 3. Results

Whatever the injection route, the results (safety, tolerability and immunogenicity) did not show any significant variation regarding the position of the injection site either on the forearm or on the deltoid. Therefore for each route, the data were pooled by area (forearm, deltoid).

### 3.1. Safety and tolerability evaluation

#### 3.1.1. Pain intensity at needle insertion and at products injection

The results indicate a significant decrease of pain at needle insertion using IDJ compared with IDS and IM ($p < 0.0001$) (Fig. 2a). Vaccine injection with IDJ generated less pain compared to both IDS ($p = 0.0028$) and IM ($p = 0.0001$) (Fig. 2b). No difference in pain intensity was observed following saline solution injection between IDJ and IDS. Pain at saline solution injection by both IDJ and IDS routes were significantly higher when compared to IM ($p < 0.0001$) (Fig. 2c).

#### 3.1.2. Reactogenicity evaluation of local SolAE

There was no significant difference regarding the occurrence of pain after vaccine injection between IDJ and IDS over the reactogenicity period. Compared to both IDJ and IDS, IM injection of vaccine induced more frequently pain the evening after injection ($p = 0.0001$) (Fig. 3a). There was no significant difference regarding the occurrence of pain after saline injection between IDJ and IDS over the reactogenicity period; they both induced pain very rarely. Compared to both IDJ and IDS, IM injection of saline solution induced pain more frequently 30 min ($p = 0.0007$ and $p = 0.0026$) and the evening ($p < 0.0001$ and $p = 0.0001$) after injection (Fig. 3b).

There was no significant difference regarding the occurrence of redness after vaccine injection between IDJ and IDS. They both frequently induced redness during the reactogenicity period. IM

injection of vaccine induced no redness during the reactogenicity period (Fig. 4a).

IDJ injection almost always induced redness 30 min after saline solution injection; the difference with both IDS and IM was highly significant ($p < 0.0001$). For the other time points, the 3 routes were similar and induced redness after saline injection less frequently compared to vaccine injection (Fig. 4b). It is noticeable that the redness at 30 min with IDJ is more frequent after saline solution injection compared to vaccine injection.

There was no significant difference regarding the occurrence of pruritus after vaccine injection between IDJ and IDS. The maximum occurrences of pruritus occurred at Day 1 and Day 2. IM vaccine
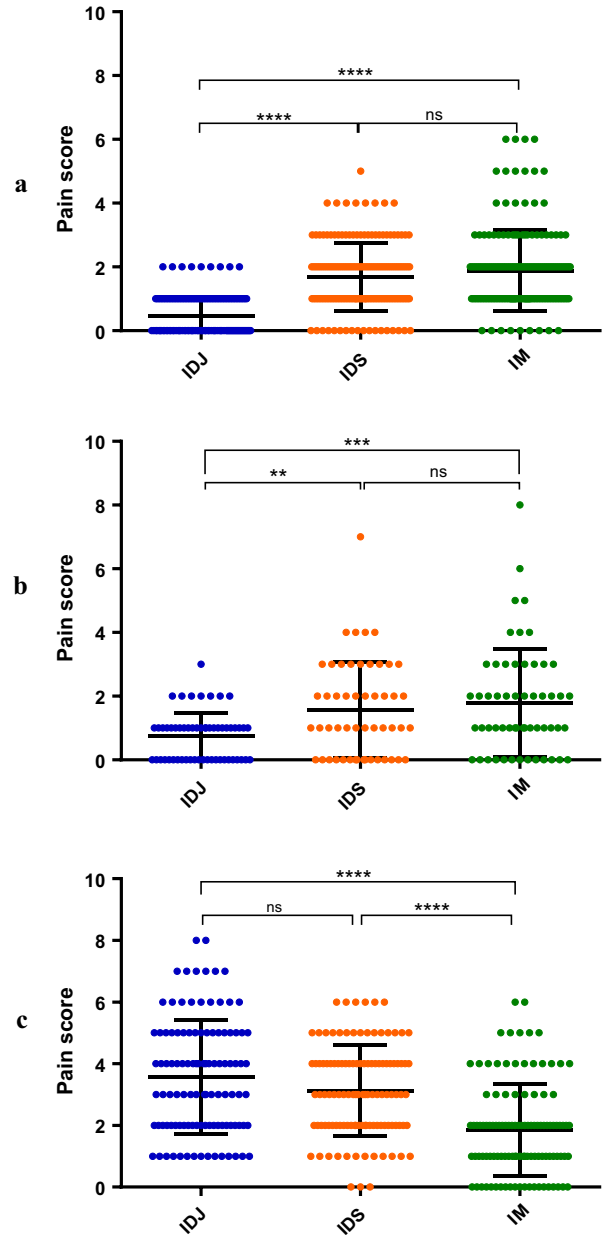


**Fig. 2.** (a): Pain score at needle insertion for the 3 routes (all sites pooled, number of insertions: $165 \times IDJ$, $165 \times IDS$, $165 \times IM$, ****: $p < 0.0001$). (b): pain score at vaccine injection for the 3 routes (all sites pooled, number of injections: $57 \times IDJ$, $52 \times IDS$, $56 \times IM$, **: $p = 0.0028$, ***: $p = 0.0001$). (c): pain score at saline solution injection for the 3 routes (all sites pooled, number of injections: $108 \times IDJ$, $113 \times IDS$, $109 \times IM$, ****: $p < 0.0001$). Pain score: volunteers' self-evaluation based on the Visual Analog Scale from 0 to 10. Mean value +/− SD per group. Mann-Whitney test. *ns* = not significant.
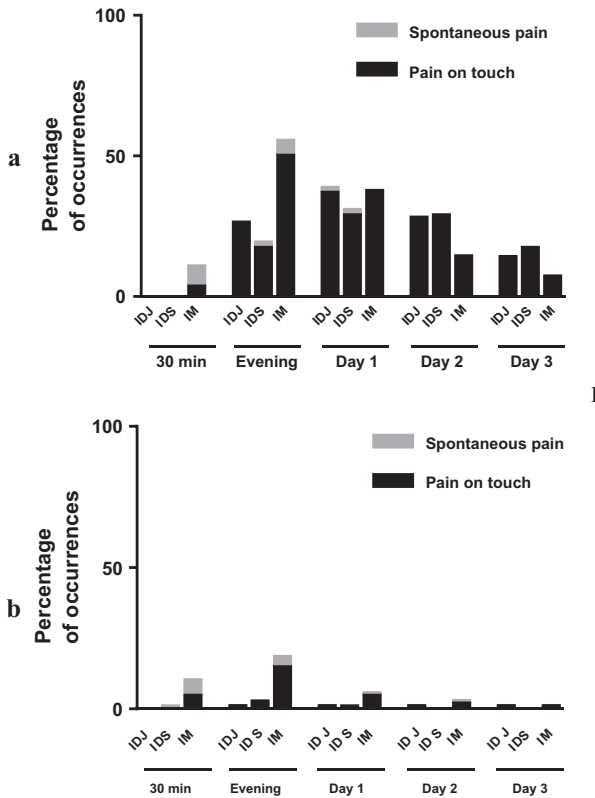
**Fig. 3.** (a) Pain following vaccine injection with IDJ, IDS and IM at different time points (all sites pooled, number of injections: 57 × IDJ, 52 × IDS, 56 × IM). (b) Pain following saline solution injection with IDJ, IDS and IM at different time points (all sites pooled, number of injections: 108 × IDJ, 113 × IDS, 109 × IM).

injection induced no pruritus during the reactogenicity period (Fig. 5). Whatever the route, saline solution injection induced no significant pruritus.

### 3.1.3. Reactogenicity evaluation of systemic SolAE

Forty-one systemic SolAE were recorded in the overall study. Headache was the most common systemic SolAE recorded in the study, followed by gastrointestinal disorders, asthenia, dizziness, malaise, nausea, myalgia and fever.

18 systemic SolAE were related to the vaccine. No systemic SolAE was assessed as related to the devices.

### 3.1.4. Reactogenicity evaluation of Unsolicited AE

Ninety-eight Unsolicited AE have been reported from 43 subjects during the study. 21 participants did not present any Unsolicited AE. 83 Unsolicited AE were not related to the study, 9 were related to the vaccine, 4 to IDS and 2 to IM. Hematomas were reported as related to ID or IM classical syringes. No Unsolicited AE was related to DebioJect™. No Serious Adverse Event (SAE) was reported during the study.

### 3.2. Immunogenicity evaluation

The second objective of this study was to evaluate immunogenicity of the IDJ route compared to IDS and IM routes. None of the subjects had detectable rabies VNA before receiving the first dose of vaccine. All study participants were above threshold of protection (0.5 IU/ml) at last study visit, 56 days after first vaccination (Fig. 6a). Consequently, all participants were considered as properly immunized against rabies at the end of the study. In addition, no significant differences between groups were observed in rabies
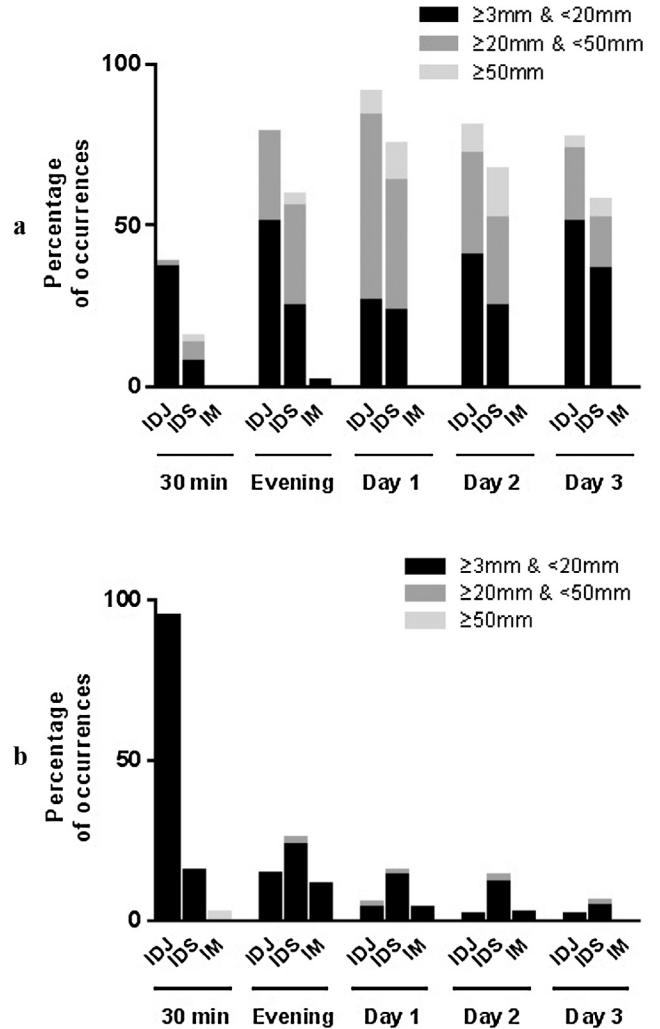


**Fig. 4.** (a) Redness following vaccine injection with IDJ, IDS and IM at different time points (all sites pooled, number of injections: 57 × IDJ, 52 × IDS, 56 × IM). (b) Redness following saline solution injection with IDJ, IDS and IM at different time points (all sites pooled, number of injections: 108 × IDJ, 113 × IDS, 109 × IM).
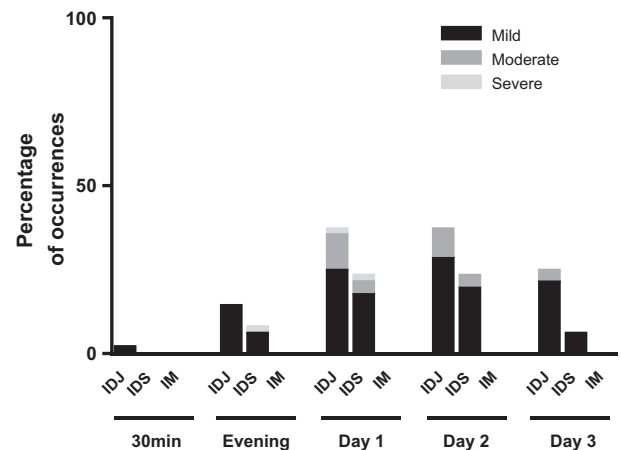


**Fig. 5.** Pruritus following vaccine injection with IDJ, IDS and IM at different time points (all sites pooled, number of injections: 57 × IDJ, 52 × IDS, 56 × IM).

VNA titers at any study visit (Fig. 6b). In this study, the humoral immune response following rabies vaccine administration with IDJ (0.1 ml) was similar to IDS (0.1 ml) and IM (0.5 ml).
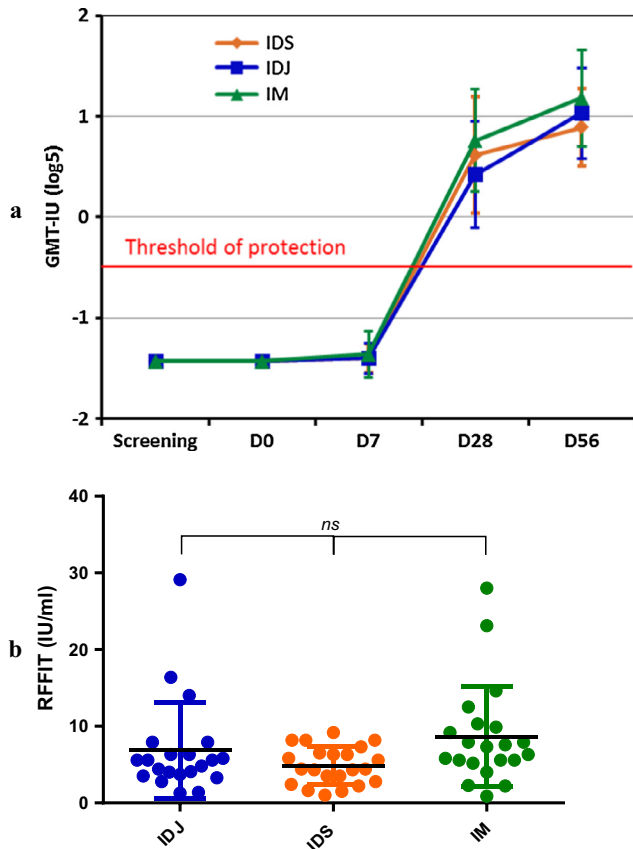
**Fig. 6.** (a) Geometric Mean Titers (GMT) calculated after logarithmic transformation (log 5) of antibody titers, per group at screening, first vaccination (D0), second vaccination (D7), third vaccination (D28), and 56 days after the first vaccination (D56). Error bars represent 95% confidence intervals. Threshold of protection: Log$_5$(0.5) ∼ −0.43. (b) Comparative assessment of rabies titers in Group A, B or C at day 56. Graph represents individual or mean value +/− SD of rabies virus neutralizing titers (IU/ml) per group (21xIDJ, 22xIDS, 21xIM).

## 4. Discussion

An essential aspect for successful commercial use of intradermal injection tools is the guarantee of complete, reproducible and user-independent insertion of the microneedle within the targeted tissue. Incomplete insertion may lead to leakage during injection, limiting the accuracy of the dose to be delivered. The skin behaves like a visco-elastic medium [30] which deforms when a force is applied on its surface. Depending on the injection site, this deformation may vary between a few tens of micrometers up to a few millimeters. In any case, it will be at least similar to the length of the micro-needle and may therefore prevent it from penetrating the skin. This issue is obviously not present in IDS injections, where the length of the needle is large compared to the skin deformation. One way to facilitate tissue penetration is to limit the contact surface of the microneedle with the tissue, increasing the locally applied pressure proportionally [31]. This is the approach used in the Becton Dickinson's Soluvia system where the needle is located in a cavity, limiting the contact surface to the needle only, the depth of penetration being controlled by walls present around the needle [32]. Another approach, based on the viscous nature of skin, consists of supplying the microneedle with sufficient velocity that the skin doesn't deform significantly when impacted [33], allowing a full penetration.

After insertion of the needle into the skin, the liquid solution can be delivered. Typical amounts of fluid that are delivered represent a volume that will induce local deformation of the skin creat-

ing the well-known bleb [32]. In order to allow bleb formation, it is necessary to limit the pressure applied on the tissue during injection. Too high pressure applied to the skin may increase its fluidic resistance and limit its ability to absorb fluid [34,35]. On the other hand, some pressure still needs to be applied during injection to maintain the microneedle within the skin. The inserter used in this study has been designed to reconcile these two behaviors. The concern of applying too high pressure during the injection led the experimenters in the first series of healthy volunteers to slightly pull the inserter during injection leading to leaks or partial leaks in about 30% of cases. After improvement of the material training stressing the fact that the inserter had to be maintained firmly in place, the success rate went up to 98% (the remaining 2% of failure were due to an operator handling error during device preparation).

A crucial part of the present trial was the assessment of comparative pain perceived by the volunteers using the three different injection methods: IDJ, IDS and IM. Particular attention has also been paid to distinguish between the pain felt at the insertion of the needle into the skin, which seems mostly related to the design of the device and its physical interaction with the tissue, from the pain felt at injection, which may be strongly influenced by the chemical formulation of the injected substance.

The pain felt at the insertion site of the needle was significantly lower for IDJ than for both IDS and IM routes. This is compatible with the lower invasiveness of the former method where a very short needle is introduced within a few milliseconds. By using IDS, the healthcare provider may puncture back and forth repeatedly to find the right spot for injection whereas the needle has to cross several layers of tissue using IM injection. Pain induced by saline solution injection was comparable in IDJ and IDS, both inducing more pain than IM injection. This might be explained by the mechanical constraints in the tissues due to the volume of liquid delivered, being higher in the skin compared to muscle, which induces more tissue damage [3]. It is noticeable that although the pain at IM injection was similar in vaccine and saline solution, the pain at vaccine injection was significantly lower compared to saline injection in both IDS and IDJ. This might be explained by a specific effect of the vaccine at IDJ and IDS injection sites which was absent in IM injection. With both IDJ and IDS, pain after injection was only due to the vaccine product itself, whereas pain was negligible with saline solution indeed. In some cases, IM injection of saline solution induced pain during the reactogenicity period. This may have been due to mechanical constraints induced in muscles.

IDJ injection of saline solution almost always produced redness 30 min after injection. This might be explained by the reduced depth of injection or by the mechanical impact of the microneedle to the skin due to its penetration speed. This effect seemed to be reduced by the vaccine. In contrast, for the following time points with IDJ and IDS, redness frequency and severity were usually higher with vaccine compared to saline. For both IDJ and IDS, the vaccine itself had an effect on redness. For vaccine and saline solution, IM injection almost never induced redness or pruritus, which might be explained by the depth of the injection.

With IDJ and IDS, pruritus after injection was only due to the vaccine product itself.

## 5. Conclusion

In accordance with the very small size of its needle, DebioJect™ induced significantly less pain at needle insertion than IDS and IM. It is also noticeable that injection using DebioJect™ was significantly less painful compared to IDS and IM injections during rabies vaccination.

A reduced dose of rabies vaccine (in this case, 0.1 ml instead of 0.5 ml) administered with IDJ induced a humoral immune

response similar to a reduced dose delivered by IDS or to a full dose delivered by IM. This result is in accordance with previously published clinical data [1].

This study clearly shows that the DebioJect™ device is safe, well tolerated and able to deliver at least 100 μl of vaccine into the dermis with high reliability and accuracy. Further studies need to be conducted in order to confirm these features in different populations, e.g. elderly or pediatric. In addition, improved immune responses may be expected due to the very precise control of the depth of injection allowing administration of the vaccine in the best-suited areas regarding the abundance of the targeted immune effectors.

Considering the drawbacks of the Mantoux method when performed by healthcare workers without high level of expertise as in this study, DebioJect™ offers the potential for fully studying and developing this route of injection and its benefits like dose sparing.

## Acknowledgements

## Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.vaccine.2016.09.069.

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