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COMMUNICATION

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COMMUNICATION

Potential remote drug delivery failures due to temperature-dependent viscosity and drug-loss of aqueous and emulsion-based fluids

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Abstract: The ability to inject wild animals from a distance using remote drug delivery systems (RDDS) is one of the most effective and humane practices in wildlife management. Several factors affect the successful administration of drugs using RDDS. For example, temperature-dependent viscosity change in aqueous (Newtonian) or water-in-oil emulsion (non-Newtonian) fluids, commonly used in tranquilizer and adjuvant-based vaccines, respectively, can potentially result in drug delivery failure. To better understand impacts due to viscosity changes, we investigated the fluid dynamics and ballistics involved in remote drug delivery. Our research was divided into two phases: we investigated the viscosimetric physics in the first phase to determine the fluid behavior under different temperature settings, simulating recommended storage temperature (7ºC), plus an ambient temperature (20ºC). In the second phase of our study, we assessed the drug delivery efficiency by specialized darts, using a precision CO_2 projector and a blowgun. Efficiency assessment was done by comparing the original drug volume with the actual volume injected after firing the dart into a fresh pork hide mounted on a ballistic gel. Before testing, we configured the required minimum impact velocity as our parameter for intramuscular injection (determined as > 40 m/sec). All executed dart-deployments performed satisfactorily, despite initial concerns of potential incomplete drug delivery, however, noteworthy drug loss was observed (>10%) associated with drug residues in syringe/dart dead space and within the transfer needle. This could potentially result in inaccurate dosing depending on the drug used. Furthermore, the use of a blowgun for remote drug delivery (>3m) is discouraged, especially when using specialized darts, as the required minimum dart velocity for adequate penetration is difficult to reach, in addition to a loss of precision during targeting.

Keywords: Ballistic, darts, inject, immunogenic, remote drug delivery system, tranquilizer, vaccines, wildlife.

Abbreviations: RH—Efficiency assessment of remote drug delivery systems.

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Competing interests: The authors declare no competing interests.

For **Author details**, **Author contribution** and **Portuguese abstract** see end of this article.

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INTRODUCTION

The ability to inject wild animals from a distance without the need for physical restraint has many advantages, including logistics, safety, and improved animal welfare. Several factors, however, influence the success of drug delivery using a remote drug delivery system (RDDS), including the user's ability to accurately use RDDS (Cattet et al. 2006), ambient influences (weather/light), dart ballistics, and drug solution characteristics. The drug's specific fluid characteristics can potentially interfere with the delivery efficiency when used in a dart and can be easily overlooked, especially when it comes to temperature-dependent viscosity (Evans et al. 2015). This is an important issue for wildlife vaccines. Vaccines developed for wildlife applications have to be highly immunogenic, thus permitting a single dose application. The duration of immunity after vaccination should also last long. Water-in-oil emulsions and adjuvants provide such attributes, but depending on temperature, the emulsion's viscosity (fluid resistance) can be a hindrance if remote drug delivery is to be used for vaccine delivery.

RDDS technologies have progressed significantly in recent times, allowing for specific application needs. For example, a vaccine with the aforementioned characteristics has to be injected as a bolus (depot), subcutaneously or intramuscularly. This can be accomplished with a single-port cannula dart. On the other hand, a tranquilizer drug is delivered using a dart with a tri-port cannula that injects the drug over a large area in a dispersed fashion, facilitating quicker drug absorption for faster biological effects.

The work described in this paper was motivated by an experience during our immunocontraception project for wildlife population control. While administering the immunocontraceptive vaccine stored at 4–7 ºC intramuscularly by a hand syringe, a substantial manual force was required even with a large diameter cannula (18 gauge). We were, therefore, concerned about potential delivery failure or incomplete delivery with RDDS. A potential solution was to increase the temperature of the vaccine to ambient temperature before deployment. This study aimed to assess the impact of temperature on the delivery of a solution while using RDDS. Although there are several publications on the use of darts in wildlife (Kreeger 1997; Cattet et al. 2006; Cracknell 2013; Evans et al. 2015; Griffin 2015; McCaan et al. 2017; Rosenfield 2017), to our best knowledge, none addressed the fluid dynamics and efficiency of drug delivery. Furthermore, we investigated if specialized darts for vaccine delivery can be adequately deployed by blowguns.

Objectives

1) To assess temperature-dependent viscosity dynamics of aqueous and emulsion solution by comparing Newtonian (aqueous) and non-Newtonian (emulsion) fluid behavior under the influence of temperature variance on their viscosity dynamics.

2) To determine minimum impact velocity and dart delivery ballistics by classifying the minimum impact velocity (MIV) necessary for adequate dart penetration, to minimize potential tissue damage using:

a. CO_2 projector (20m)

b. Blowgun (3m)

3) To evaluate drug delivery efficiency of aqueous and emulsion-based solutions at two different temperature conditions (storage 7ºC; ambient 20ºC), by comparing weights of the syringe, transfer needle, dart, and dart cannula before and after use/deployment to identify any potential drug volume loss.

MATERIALS & METHODS

Experimental design for fluid behavior of emulsion and aqueous solution:

To determine the impact of temperature on the viscosity of aqueous and emulsion-based fluids, as typically found in injectable anesthetics and vaccines, we used the programmable Rheometer, Brookfield DV-III, a cone plate version viscometer, and the Waele's Ostwald equation:

$$\tau = K\left(\frac{\partial u}{\partial y}\right)^n$$

whereby:

• K (flow consistency index) expressed in (N/m ^ 2). (S^n)

• n (flow behavior index), dimensionless.

• du / dy (shear rate), expressed in 1/s.

Water-in-oil emulsion (non-Newtonian fluid)

We analyzed the temperature impact on the viscosity of an injectable, water-in-oil based emulsion. We used an original sham vaccine (USDA, NWRC, Fort Collins, USA) at two temperature settings. First, simulating the manufacturer's recommended storage temperature of 7ºC, and second, at an ambient temperature of 20ºC. The temperature of the tested fluid was maintained by using the Rheometer's temperature-controlled circulating bath. Subsequently, the viscosity was measured with different spindle sizes and rotation velocities. Each test

was then repeated.

Aqueous solution (Newtonian fluid)

For comparison, we also tested an aqueous-based fluid, simulating a drug consistency commonly found in tranquilizer drugs by preparing a saline/ethylene glycol solution (90% v/v to 10% v/v). The viscosity tests were performed at 20ºC. Subsequently, the viscosity was measured with different spindle sizes and rotation velocities. Each test was then repeated.

Statistics

To evaluate the differences between the means for viscosity samples, a bi-caudal Welch's t-test was used considering unequal sample sizes. The data was analyzed using Stats Package (Version 3.6.2) in R (R Core Team 2020).

Dart-delivery assessment

Equipment

CO_2 Projector: Distance darting was performed using a high-precision CO_2 projector (X-Caliber, 50 cal. [12.3mm] Pneu-Dart, Inc., Williamsport, PA, USA) with a mounted scope.

Blowgun: For the blowgun tests, we used a 58.5cm length blowgun, with a 12.3mm diameter (Pneu-Dart, Inc.).

Darts (n=6): The employed dart specifications: Type P, cannula length 31,75mm, gel collar, single-port, and a tri-port, with an explosive charge.

Chronographer: Dart-velocity recording was performed by using a precision chronographer, recorded in m/sec (accuracy +/- 0.25%), along with external digital data recording (Ballistic Precision Speed Chronograph, Caldwell, USA). The chronographer was placed 30cm in front of the target field. The darts were fired in such a way, that they pass two screens, and the time it takes for the darts to travel the distance between the screens is measured electronically.

Target: The target was a 112mm thick piece of fresh pigskin with an intact layer of adipose tissue and some visible areas of connected muscular tissue, serving as an indicator for intramuscular (IM) injection (Image 1). The pigskin was mounted onto a block of 10% ballistic gel.

Basic set-up - Distance darting: The CO_2 projector was mounted on a rifle shooting rests (Caldwell, USA), with the scope zeroed-in at a distance of 20m.

Shooting execution: Using the manufacturer's guidelines for initial pressure settings on the CO_2 projector, we developed our own settings, designated as "minimum impact velocity" (MIV). Most common dart delivery-failures are due to inadequate pressure settings on the CO_2 projector. Too low of a pressure and the dart will not reach their intended targets or bounce off the animal. Too high of a pressure, and the dart may provoke extensive tissue injury or ricochet. MIV refers to the lowest functional pressure setting, allowing for the dart to reach its target with adequate intramuscular penetration, believed to minimize tissue damage. Optimal depth was considered when the dart's gel collar was positioned on the far side of the pork skin's adipose tissue, allowing for the cannula orifice to reach muscle tissue. This was accomplished by gradually increasing the gas pressure and recording the dart's velocity. The results were quantified by needle penetration depth:

* Full = gel collar past skin/fat layer
* ½ = gel collar stuck within the skin/fat layer
* F = failure of gel collar to penetrate the skin

The setting with the lowest pressure that resulted in full penetration was used as the new MIV.

Basic set-up - Blowgun

Darting via blowgun was performed from a distance of 3m, using the same chronograph setup to assess minimum impact velocity.

Assessment of Drug Delivery Efficiency

Before testing, all syringes, transfer needles, and darts were identified with permanent markers. The efficiency of drug delivery was determined by the weight-differences between:

1) original quantity (1mL) of the sham vaccine, prefilled in 3mL syringes (Henke-Sass, Wolf GmbH, Germany)

2) 18G x 76.2mm transfer needle (Pneu-Dart, Inc.), before and after use

3) Dart empty weight

4) Full-filled dart weight (dart + drug load) prior to deployment

5) Dart weight after deployment (drug injected into the target)

Weight differences were determined by using a digital precision top scale (500g x 0.01g).

The difference in weight between the original sham vaccine-loaded syringe and the weight of the vaccine-filled dart after deployment was considered the net drug weight deposited. Any weight difference not being equal to the 1mL sham vaccine weight was considered to be the amount of drug lost.

RESULTS

Fluid behavior

Water-in-oil emulsion (non-Newtonian fluid)

The rheological behavior of the sham vaccine, a water-in-oil emulsion, indicated that there is no linear relationship between tension and strain rate. It is, therefore, a liquid with non-Newtonian behavior. As for the temperature, it was observed that the increase in temperature implies a greater fluidity of the emulsion, a behavior that is typical of viscous liquids (Fig.1). The emulsion's viscosity was significantly different at two different temperatures, 7ºC vs. 20ºC (Bi-caudal Welch's t-test [p=0.04052;95% CI, 598.00–20160.65]).

Aqueous Solution (Newtonian fluid)

The aqueous solution presented a linear relationship between the stress and the rate of deformation, i.e. Newtonian behavior. Therefore, the viscosity of the aqueous solution can be defined as being constant at a temperatures of 7ºC, and constant at a temperature of 20ºC, however, significantly different when compared to one another, (viscosity cP 8 to cP 2, respectively), (Fig. 2) (Bi-caudal Welch's t-test [p=0.0000156; 95% CI,-7.634565/-5.850435]).

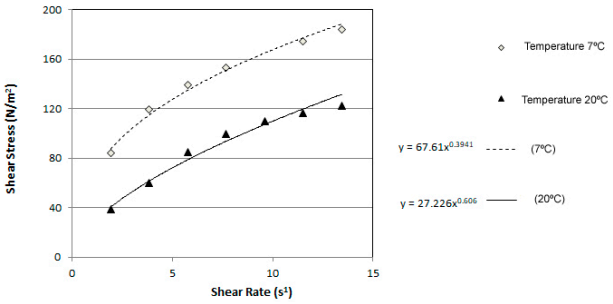

Figure 1. Shear stress test of the emulsion-based solution at different temperatures. The emulsion's viscosity was significantly different at two different temperatures, 7ºC vs. 20ºC (Bi-caudal Welch's t-test [p = 0.04052; 95% CI, 598.00 – 20160.65]).

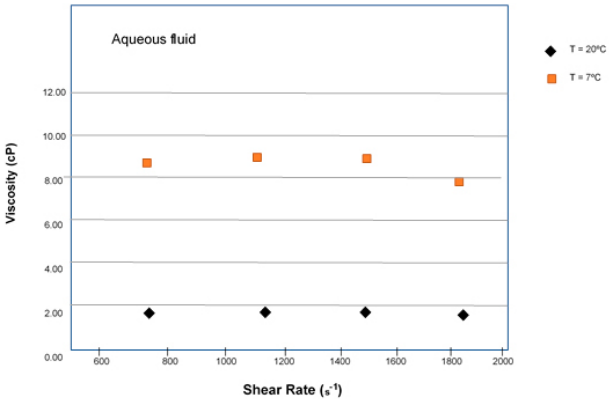

Figure 2. Shear stress test of the aqueous solution at different temperatures. The viscosity of the aqueous solution was also significantly different at two different temperatures, 7ºC vs. 20ºC (Bi-caudal Welch's t-test [p = 0.0000156; 95% CI, -7.634565 / -5.850435]).

Minimum impact velocity

For our specific equipment, the pre-determined impact velocity necessary for adequate dart cannula penetration to reach intramuscular tissue was ≥ 40m/s. As demonstrated in Images 1 & 2, the identified MIV allowed all deployed darts to reach IM injection depth.

Overall drug delivery quality

Not considering drug volume loss due to cannula/syringe/dart dead-space, the drug volume deposited of all deployed darts were satisfactorily (Image 3). Images 4 A and B demonstrate the different deposit characteristics with a single-port and a tri-port cannula, respectively.

Dart weight-differences

We identified a mean drug weight difference between original drug volume (1mL pre-deployment) and injected volume (0.886mL) post-deployment). The weight difference of 0.114gm was statistically significant (p<0.01; 95% CI, 0.076–0.123).

Weight Difference of deposited drug volume by temperature variations:

Differences in deposited drug volume due to temperature variant (7°C vs. 20°C) were considered statistically not significant (p=0.3194).

Drug Volume Loss

The only significant drug loss identified was related to the residue in the transfer needle in addition to the residue in the syringe hub (dead space), (Image 5), a space between the syringe needle and barrel.

Hypothesized drug loss using dead space's volume calculations for transfer syringe/needle and dart/canula

Formula: $V = \pi r^2 h$

Where the 18G syringe needle volume (dead space) = 0.84 mm ID x 76.2 mm length.

$V = 4.22 mm^3 = 0.00422 mL$

Where the 14G Dart cannula = 1.6mm ID x 31.75mm

$V = 67.06 mm^3 = 0.06706 mL$

Total syringe needle/dart canula residue volume: 0.07128mL

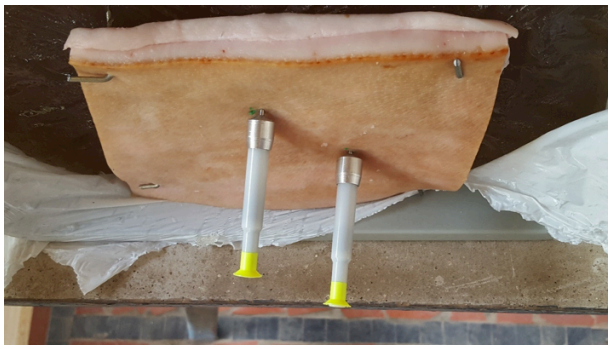

Image 1. Darting – Drug deposit evaluation. Pork hide mounted on a block of ballistic gel with two darts showing full penetration of the dart's cannula. © D. Rosenfield

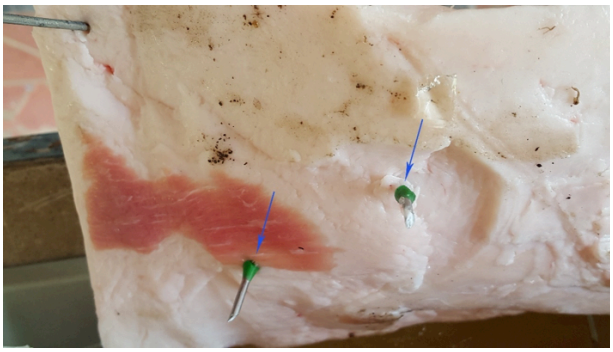

Image 2. Showing the backside of the pork hide, adequate penetration of the dart cannulas, with the single-port reaching muscle tissue, secured by the gel collar past the adipose tissue (blue arrows). © D. Rosenfield

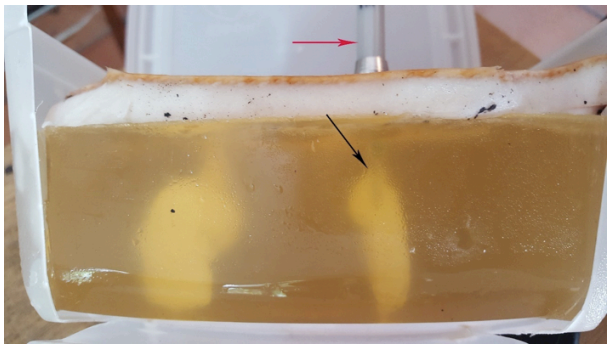

Image 3. Birdeye view of the ballistic gel with mounted pork hide. Depicting the deployed dart (red arrow), and the quality of the drug deposits in the form of clouds within the ballistic gel (black arrow). © D. Rosenfield

Also, prior identified dead space of conventional 1mL syringes (Küme et al. 2012; UC Davis 2016) of 0.066mL, including the same volume amount for the dart hub, the total hub volume projected is 0.132mL. Adding the calculated syringe/cannula and the hub dead space, the total residue volume could be as high as 0.203mL, a potential 20% drug loss.

DISCUSSION

The initial concern due to the potential increase in fluid resistance for an emulsion-based drug stored at 7ºC was corroborated during the viscosity assessment, where temperature-dependent rheological behavior was evident. Similar concerns were reported by Baker et al. (2005) and Kirkpatrick et al. (2011), when observing delivery failure, potentially linked to the viscosity issue of a polymer mixture. Our experiment results confirmed a significant temperature impact on emulsion-based fluids. Specifically, that a decrease in solution temperature increases fluid resistance (Palm et al. 2015).

Although drug delivery using darts was efficient for fluids at 7ºC as well as 20, as observed in our pork skin/ballistic gel setup, attention should be paid to other potential delivery failures when darting live animal, in particular, due to drug fluids traveling back the wound channel, as described by (Evans et al. 2015). The use of a blow-gun is discouraged as the indicated minimum impact velocity of specialized darts for adequate perforation would be difficult to achieve, leading to inadequate injection depth, failure to trigger propellent mechanism or bounce-backs.

Finally, drug loss due to accumulated residue in the syringe and dart dead spaces, dart cannula and transfer needle should be considered when using drugs sensitive to minute variations. To the best of our knowledge, scientific literature on potential drug loss due to dead-space in darts does not exist, however, similar corroborated information can be found in human medicine (Bobashev & Zule 2010; UC Davis, YSP, 2016).

CONCLUSION

Contrary to our initial concerns, the findings of this study demonstrated efficient drug deliveries, without the need to warm an emulsion-based vaccine to ambient temperatures. Nevertheless, the drug volume loss attributed to dead-space residues of the syringe, needle, dart during drug transfer from the syringe to dart, is noteworthy. Drug delivery with specialized darts, using any kind of propellant, will bring about tissue damages to a certain degree. But risks associated with physical restraint (nets, traps, etc.) are much greater.

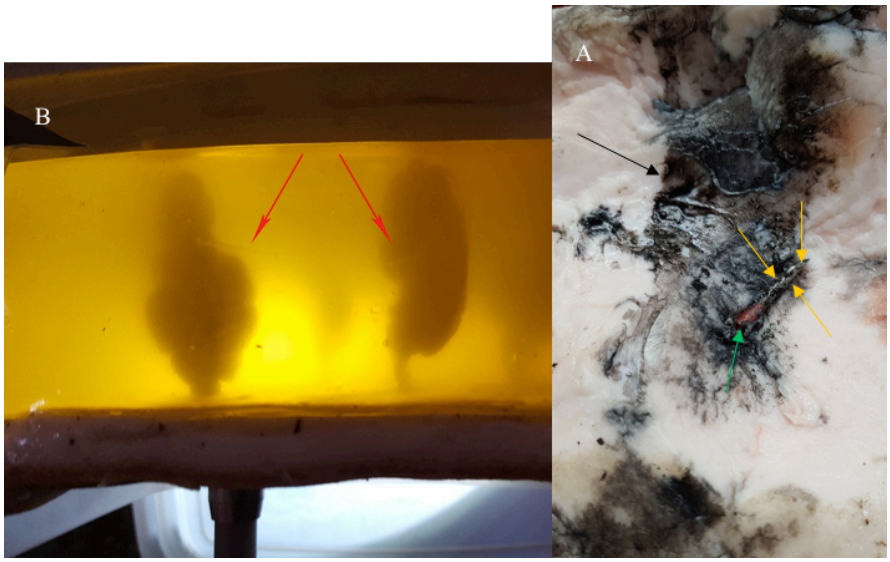

Image 4, A—Emulsion deposits (red arrows) by dart injection, using background-light to enhance contrast | B—For comparison, dispersed injection of a colored aqueous solution with a tri-port dart. Orange arrows: cannula's orifices, green arrow: cannula with gel collar, black arrow: injected colored aqueous solution. © D. Rosenfield

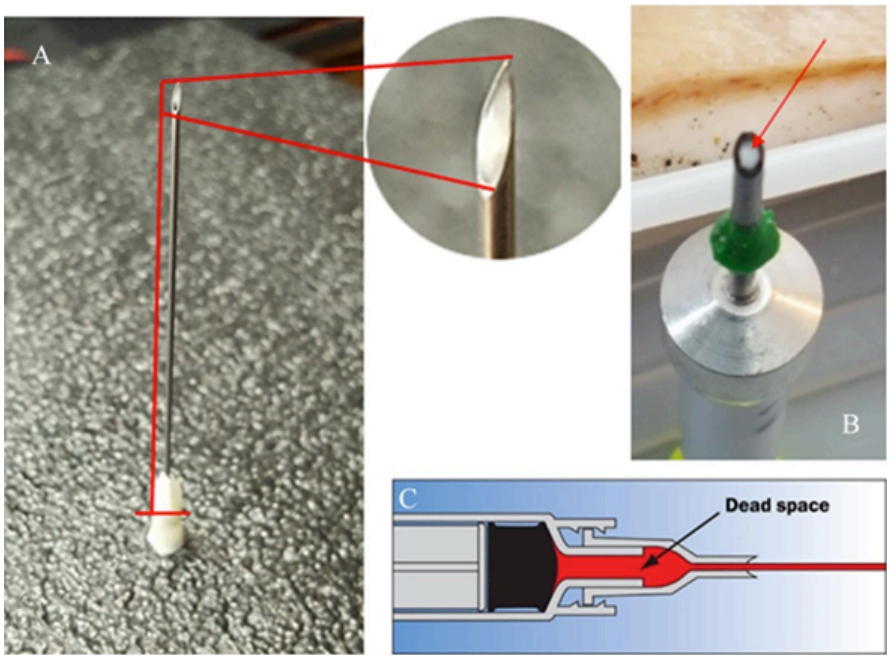

Image 5. Dead space evidence, post-application. A—red indicator, residue in a 76.2mm length transfer needle dead space | B—drug residue in the dart's 31.75mm cannula | C—illustrated dead space (UNC, 2018). © D. Rosenfield

Remote drug delivery systems, with their high precision, reliability of drug delivery, and safety for animals and personnel, may outweigh the potential adverse effects. Overall, our results suggest that RDDS can be used for emulsion-based drug delivery.

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Spanish Resumen: La habilidad para inyectar animales salvajes a distancia, utilizando sistemas de administración remota de medicamentos (RDDS), es una de las más efectivas y humanizadas prácticas en el manejo de la vida silvestre. Varios factores afectan la administración exitosa de medicamentos usando RDDS. Por ejemplo, el cambio de viscosidad, dependiente de la temperatura en fluidos acuosos (newtonianos) o las emulsiones de agua en aceite (no newtonianos), comúnmente usados en tranquilizantes y en vacunas con base de adyuvantes; estos cambios pueden potencialmente resultar en fallas en la administración de los fármacos. Para comprender mejor los impactos debidos a los cambios de viscosidad, investigamos la dinámica de fluidos y balística involucrados en la administración remota de fármacos. Nuestra investigación se dividió en dos fases: en la primera fase investigamos la física viscosimétrica para determinar el comportamiento del fluido a diferentes niveles de temperatura, simulando la temperatura de almacenamiento recomendada (7ºC), además de una temperatura ambiente (20ºC); en la segunda fase, evaluamos la eficacia de la administración de fármacos mediante dardos especializados utilizando un rifle de precisión de CO2 y una cerbatana. Se realizó una evaluación de la eficiencia comparando el volumen de fármaco original con el volumen real inyectado después de disparar el dardo en una piel de cerdo fresca montada en un gel balístico. Antes de la prueba, configuramos la velocidad de impacto mínima requerida para nuestros parámetros y la inyección intramuscular (determinada como > 40 m/s). Todos los despliegues de dardos se comportaron satisfactoriamente, a pesar de las preocupaciones iniciales de una posible entrega incompleta del fármaco. Sin embargo, se observó una pérdida de fármaco notable (~ 10%) asociada al residuo de fármaco en el espacio muerto de la jeringa / dardo y dentro de la aguja de transferencia. Esto podría potencialmente resultar en una dosificación inexacta dependiendo del medicamento utilizado. Por otra parte, el uso de la cerbatana para administración remota de medicamentos (> 3 m) es desaconsejada, especialmente cuando se utilizan dardos especializados, debido a que la velocidad mínima requerida para una penetración adecuada es difícil de alcanzar, además de la pérdida de precisión al apuntar.

Palabras clave: balístico, dardos, inyectable, inmunogénico, sistema de administración remota de fármacos, tranquilizante, vacunas, vida silvestre.

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