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Comparison of different identification systems in Iberian ibex (*Capra pyrenaica*) for the control of wild populations and the traceability of game products

Comparación de diferentes sistemas de identificación para el control de poblaciones salvajes y la trazabilidad de los productos de caza en cabra montés (Capra pyrenaica)

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Mentored by
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The present work, entitled “Comparison of distinct identification systems in Iberian ibexes (*Capra pyrenaica*) for the control of hunting populations and the traceability of game products”, corresponds to the module Trabajo de Fin de Máster on the “Màster de Qualitat d’Aliments d’Origen Animal” of the Universitat Autònoma de Barcelona (UAB), valued at 15 ECTS credits, and has been mentored by Dr. Gerardo Caja López (Grup de Recerca en Remugants, Departament de Ciència Animal i dels Aliments, UAB), Dr. Gregorio Mentaberre García (Servei d’Ecopatologia de Fauna Salvatge, Departament de Medicina i Cirurgia Animals, UAB) and Dr. José Enrique Granados Torres (Departamento de Medio Ambiente, Junta de Andalucía). The direct supervision or guardianship has been carried out by Dr. Gerardo Caja of the Departament de Ciència Animal i dels Aliments of the UAB.

Bellaterra (Barcelona), 1 of September of 2019,

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LIST OF ABBREVIATIONS

aRFID	Active radio-frequency identification.
e-ID	Electronic identification.
e-IM	e-ID by intramuscularly injected transponder between the scapuli.
e-IP	e-ID by intraperitoneally injected transponder.
e-RB	e-ID by ceramic rumen bolus with transponder.
e-SUB	e-ID by subcutaneously injected transponder in the armpit.
FDX-B	Full duplex B interface radio-frequency technology.
HDX	Half duplex interface radio-frequency technology.
PTB	Ports de Tortosa-Beseit.
RFID	Radio-frequency identification.
SNNS	Sierra Nevada Natural Space.
v-ID	Visual identification.

ABSTRACT

The efficiency of different visual and electronic identification systems was studied in 2 fenced populations of Iberian ibexes (*Capra pyrenaica*) in Tarragona and Granada (Spain), using a total of 58 individuals each of them identified simultaneously with visual ear tags or collars and other electronic devices. Electronic devices were injectable transponders of 2 sizes: small-sized (12-12.2 mm, n = 47), injected intramuscularly between the escapuli, and large-sized (32 mm), injected subcutaneously in the left armpit (n = 58) or intraperitoneally in the belly (n = 44), and ceramic rumen boluses (n = 30) administered orally and retained in the forestomaches. The study lasted 1.6 to 5 years according to devices. Except ear tags (<50%), all devices were highly retained (94 to 100%), although readability was variable and lower than retention rate (96 to 99%). Main losses were observed in large-sized transponders immediately after injection, while small-sized transponder were difficult to read because of migration. Rumen boluses showed the greatest retention rate (100%) and readability (98.6%), being applied safely in ibexes greater than 28 kg. Retention was predicted at the long term (10 yr) and using an interspecific logistic equation for ruminants (R^2 = 0.96). Rumen boluses were also safe for predators, scavengers and game consumers and recommended for traceability purposes.

RESUMEN

Se ha estudiado la eficiencia de diferentes sistemas de identificación visual y electrónica en 2 poblaciones cautivas de cabra montés (*Capra pyrenaica*) en Tarragona y Granada (España), en un total de 58 individuos, cada uno de ellos identificados simultáneamente con crotales y collares visuales y otros dispositivos electrónicos. Los dispositivos electrónicos consistieron en transpondedores inyectables de dos tamaños: pequeños (12-12.2 mm, n = 47), inyectados intramuscularmente entre las escápulas, y grandes (32 mm), inyectados subcutáneamente en la axila izquierda (n = 58) o intraperitonealmente en el abdomen (n = 44), y bolos ruminales cerámicos (n = 30) administrados oralmente y retenidos en los preestómagos. El estudio duró de 1.6 a 5 años según los dispositivos. Excepto los crotales auriculares (<50%), todos los dispositivos presentaron elevadas tasas de retención (94 a 100%), aunque la capacidad de lectura fue variable e inferior a la retención (96 a 99%). La mayoría de las pérdidas se observaron en los transpondedores grandes inmediatamente después de la inyección, mientras que los pequeños fueron difíciles de leer a causa de la migración. Los bolos ruminales mostraron la mayor tasa de retención (100%) y legibilidad (98.6%), con una aplicación segura en cabras mayores de 28 kg. La retención de los bolos fue capaz de ser predicha a largo plazo (10 años) y mediante una ecuación logística interespecífica para rumiantes (R^2 = 0.96). Los bolos ruminales fueron seguros para depredadores y carroñeros, así como también para los consumidores de carne de caza, por lo que se recomienda su uso para trazabilidad.

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1. INTRODUCTION.

1.1. Situation of the Iberian ibex population.

Iberian ibex or Iberian wild goat (*Capra pyrenaica*; Schinz, 1838) is a species of ungulates included in the *Capra Ibex* group (2n = 60) which is endemic to the Iberian Peninsula where it lives in the main mountainous systems (ranging from the sea level to 3,400 m high). It is a browser and grazer, also called a mixed feeder, depending on plant availability, and its feeding resources vary geographically, altitudinally, and seasonally. It inhabits mainly in shrub lands and rocky areas, such as inland cliffs, mountain peaks and temperate forests (Herrero and Pérez, 2008; Acevedo and Cassinello, 2009).

From the 4 subspecies of Iberian ibex reported several decades ago, there are only 2 existing currently: 1) *C. p. victoriae*, which inhabits in the center and NW of the Iberian peninsula; and, 2) *C. p. hispanica*, which lives in the Mediterranean mountains (E and S of the Peninsula), including the Ports de Beseit, Sierra de Muela de Cortés, Sierras de Cazorla y Segura, Sierras de Almijara y Tejeda, Sierra Nevada and Sierra de Ronda de Cádiz. The other 2 subspecies, *C. p. lusitanica* and *C. p. pyrenaica* (Bucardo), are now extinct since ca. 1900 and 2000, respectively (Pérez et al., 2002; Acevedo et al., 2008).

Current population is estimated as near 50,000 mature individuals (in 2002), mainly inhabiting the SE and E of the Iberian Peninsula (*Figure 1*). The largest populations are located in Sierra Nevada (Granada, 16,000 individuals), Sierra de Gredos (Ávila, 8,000 individuals) and Maestrazgo (Teruel, 7,000 individuals) as reported by Pérez et al. (2002).

The species is suffering from different threats, such as the habitat fragmentation, the illegal game, the competition with domestic goats and other introduced ungulates, and the prevalence of diverse diseases, the combination of which has been influencing the demographic changes within its population during the past decades (Colom-Cadena et al., 2014; Torres et al., 2017).

Several programs were conducted in order to stabilize or to recover local populations of *C. pyrenaica* within the Iberian Peninsula. Some successful translocations were carried out during the recent history after 1970 and mainly in the 80's and 90's (Acevedo and Cassinello, 2009), which are shown in Fig. 1 as "reintroduced".

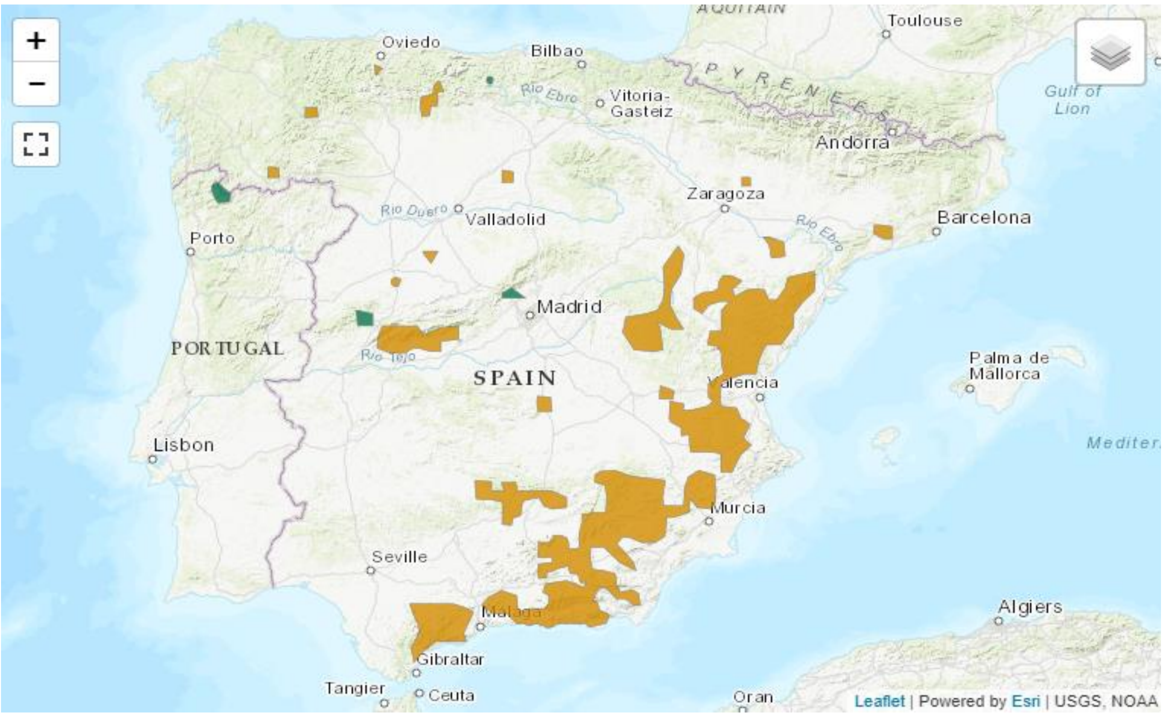

Figure 1. Map of the geographical distribution of *Capra pyrenaica* (Iberian ibex). **Brown**: extant (resident); **Green**: extand and reintroduced. IUCN (International Union for Conservation of Nature, 2008).

Nowadays, regardless the threats are still affecting *C. pyrenaica spp.*, its population is expanding, and it has been catalogued as a group of Least Concern (LC) species by the IUCN (International Union for Conservation of Nature), as reported by Herrero and Pérez (2008). The current expansion of the Iberian ibex is, most probably, due to the recovery from past disease outbreaks, the positive effects of the translocations, the decrease of hunting pressure, as well as the combination of many other factors (Acevedo and Cassinello, 2009).

1.2. Coexistence of livestock and Iberian ibexes: health concerns.

In many areas, wild and domestic goats under extensive production systems coexist. It is not a problem itself, but there are some diseases shared by both wild and domestic species. These diseases can be transmitted from the Iberian ibex to domestic populations, and vice versa, making them more prevalent and difficult to eradicate. Traceability has become of importance in some countries in order to take major control on animal health, with the aim to assure greater food safety (Rojas-Olivares, 2011).

One of the major health problems affecting the Iberian ibex is the Sarcoptic mange or "scabies" (produced by the acari *Sarcoptes scabiei*) that causes minor problems in domestic sheep and goat but has severe consequences in Iberian ibex. In the 80's scabies caused the death of about 95% of wild goats in the Sierra de Cazorla, Segura and Las Villas Natural Park, in southern Spain, and not far from there, a few years later, those of the Sierra Mágina Natural Park suffered a similar scabies episode. In 1992 Scabies mange was also detected in Sierra Nevada Natural Space, the largest population and with the greatest genetic variability of Iberian ibex (León-Vizcaíno et al., 1999; Espinosa et al., 2017).

Other relevant menace for wild goats are the Pestivirus, a group of viruses (i.e., bovine viral diarrhea or BVD) associated with hemorrhagic syndrome, abortion and fatal mucosal disease, although they have higher prevalence in domestic goats than in Iberian wild goats (Astorga-Márquez et al., 2014). On the contrary, brucellosis, which severely affects domestic goats, has a significative low prevalence in wild goats (G. Mentaberre and J. R. López, personal communication).

Despite not directly affecting human health, any disease suffered by both domestic and wild goat may influence negatively the acceptance and safety of meat products for the consumer. Since there is evidence that some illnesses can migrate or exacerbate its incidence due to the coexistence of wild and domestic goats, efficient identification systems for these wild populations are of interest in order to have an improved demographic monitoring and greater health control. Moreover, the traceability in game populations may increase the food safety of the products and their valorization for consumers, especially in the case of meat coming from protected areas such as natural parks or protected spaces.

1.3. Available methods of identification. Characteristics, pros and cons.

Animal identification (**ID**) is a practical procedure that enables to identify and track animals individually. There are many different ID systems from visual (**v-ID**) to electronic (**e-ID**), such as: skin marks, tags, collars, bracelets, injectables, rumen boluses and others.

All these ID systems have been widely studied and used in different domestic species such as goats, sheep, cattle and camelids (Caja et al., 2004), each ID method and device having its pros and cons as summarized in Table 1.

1.3.1. Visual methods.

Hot-iron and freeze branding. Red hot-iron is one of the most common traditional ID methods used in livestock (and humans) until the recent past. The individual is marked with a farm's brand, letters and/or numbers, in order to visually identify it in a permanent way by alteration of its epidermis and dermis. Nevertheless, these marks may not provide accuracy nor reliability enough: duplication, removal or alteration may occur. They are mostly used to ID the farm or the farmer, rather than individuals, so it may be not appropriate for animal traceability. Moreover, in many countries or species (i.e., horses) hot-iron is already prohibited due to animal-welfare concerns (Awad, 2016) being substituted by cold-iron branding (Caja et al., 2004). Hot-branding is still the farmer's choice for beef cattle ID in the U.S. and for bullfighting cattle ID in Spain.

Cold-iron branding consists of freezing the dermis to destroy the hair follicle pigmentation units. Similarly to hot-red, any iron code or drawing can be applied and it is mainly used to ID individuals within a farm (i.e., Holstein cows of a herd). However, it cannot be used for white haired or spotted animals, and sometimes the discoloration of the hair may be not permanent (Caja et al., 2004; Awad, 2016).

Table 1. Characteristics and pros and cons of different animal ID systems

System	Method	Efficiency	Life span	Suitability	Cost	Welfare	Main concerns
Visual	Hot branding	Low	Lifetime	Easy	Low	Low	Encoding, welfare
	Ear tattooing	Low	Lifetime	Laborious	Low	Medium	Encoding, welfare, reading
	Ear notching	Low	Lifetime	Easy	Low	Low	Encoding, welfare, reading
	Cold branding	Low	Lifetime	Medium	Medium	Medium	Encoding, coat color
	Horn marks	Low	Lifetime	Medium	Low	Medium	Encoding, poll, welfare
	Collars	Medium	Not lifetime	Easy	Low	Medium	Encoding, welfare
	Ear tags	Medium	Not lifetime	Easy	Low	Low	Encoding, losses
	Leg bands	Medium	Not lifetime	Medium	Low	Medium	Welfare, losses
Electronic passive transponders	e-IM	High	Lifetime	Easy	Medium	Medium	Migration, breakage, recovery
	e-IP	High	Lifetime	Medium	Medium	Medium	Reading, recovery
	e-SUB	High	Lifetime	Easy	Medium	Medium	Migration, recovery breakage
	e-RB	High	Lifetime	Medium	Medium	Medium	Reading
	Ear tags	High	Not lifetime	Easy	Medium	Medium	Losses
	Leg bands	Medium	Not lifetime	Medium	Medium	Medium	Welfare, losses
Electronic active transponders[1]	aRFID	High	Battery life span	Low	High	Medium	Cost, lifespan
Biometrics	Muzzle print	Medium	Lifetime	Medium	High	High	Cost, reading
	Retinal image	High	Lifetime	Medium	High	High	Cost, reading
	DNA	High	Lifetime	Medium	High	High	Cost, reading

e-IM, intramuscular; e-IP, intraperitoneal; e-SUB, subcutaneous; e-RB, ruminal bolus; aRFID, active radio frequency identification; [1]with or without sensors.

Horn marks. It consists of the hot iron branding of a series of numbers of letters directly on the horn in order to identify the animal. It is safe from fraud and easy to apply, but it is useful only in small herds and with adult animals with medium or big sized horns.

Ear notches. The animals are identified with a combination of V-shaped removed sections in specific locations of both ears (Caja et al., 2004). Every particular combination of removed areas stand for a specific number. It is a method that can only be useful for small farm ID due to the limited number of combinations possible. It can also suffer from fraud or duplication, apart from the welfare concerns (Awad, 2016).

Ear tattoos. Tattoos are based on creating series of holes that draw a code of numbers and/or letters in the skin, most usually in the internal part of the ear of the animal. After creating these small injuries, black permanent ink is applied. The ink stays in the skin, revealing the permanent code recorded. It is a traditional ID method, few invasive and distressful for the animal, but it can suffer from alteration, duplication or removal. The ID process (reading), once the animal is tattooed, may be not fast nor simple due to the location and the size of the mark or codes. The mark is supposed to be permanent, but in fact it can be altered or even removed over time (Awad, 2016). There are problems with animals having black ears (Caja et al., 2004) and the use of green ink is recommended in this case.

Ear tags. Ear tags are a single or double flag tag made of different materials (i.e., plastic or metal) which are attached to the ear of the animal, usually in the middle of the auricula. It is a visual, temporary ID system, easy to implement and to use or read. It permits a fast and simple ID of the animal and it may need no direct contact with the animal. However, this system usually gets unreadable for many reasons and can cause severe ear damages (Caja et al., 2004). Because the devices are strange bodies attached to the auricula of the animal, they can cause permanent wounds in the ears of the animal and eventually infections. In many cases the ear splits and as a consequence the ear tag may be lost. Moreover, since it is a "flag" that protrudes from the ear, the devices can be hooked with objects and ripped off the auricula causing injuries. Another cause of illegibility is the chewing of plastic ear tags, especially detected in domestic goats, that can make the numbers or even the whole tags almost disappear. Apart from losses, another reason of the low efficiency of ear tags is the human factor because the reading process is always visual and, as other v-ID systems, recorded by hand (Caja et al., 2014).

Ear tags have shown extremely variable retention rates (0.5 to 60%), depending on many factors, including the type of v-ID tag, the species, the breed and the environmental conditions (Rojas-Olivares, 2011).

Finally, ear tags have an important visual impact for both humans and other fauna when wild or domestic goats are living outdoors.

Collars. Collars are another type of visual ID, with the advantage that they are easily visible from long distances, enabling to identify animals in free range systems when marked with different colors and large numbers. There are, however, some disadvantages related to this ID devices: breakages problems and ink-vanishing, risks of hanging with forestry elements, and many others, including visual impact in wild animals and the fact that identifying young individuals requires at least the substitution of the first collar by another one few years later (2-4 years maximum) in order to avoid strangulation.

Leg bands. It consists in the application of a bracelet around one leg of the animal, at the metatarsal or metacarpal circumference region. A code is printed or written on the tag, so the animal can be identified. This ID system has demonstrated to be reliable in domestic goat (Carné et al., 2010), however it is necessary to apply the correct size of the leg tag in order not to cause injuries to the animal. Moreover, it is important to check regularly for leg damage or inflammation and to remove the leg tag if necessary, to avoid excessive constriction around the metatarsal area. All this makes the system of limited interest in wild goats.

1.3.2. Passive radio frequency identification.

Electronic ID (**e-ID**) is becoming of importance in many different areas, such as animal traceability in the food industry, ID of companion animals, and also in wild animals, as done in the present study.

During the second half of the past century there were many attempts of producing electronic devices for accurate, reliable animal ID around the world. After different initial prototypes, the first electronic successful devices used in livestock in the mid-late 80s were collars with integrated RFID (Radio Frequency Identification) passive transponders. After that, the technology evolved into many different and more sophisticated devices (Eradus and Jansen, 1999).

Transponders (transmitter-responder, microchip or ID devices), transceivers (transmitter-receiver or reading equipment), data recorder (memory) and processing software are the 4 basic components of the passive RFID systems used in animal ID. The transponder contains an integrated circuit in which an ID number is stored, and it is applied to the animal to identify. As passive devices, it does not need a battery to operate; it works with the electromagnetic energy emitted by the transceiver, which is an active device that requires a power source to operate (e.g., battery). Transceivers are composed by a radio transmitter and an antenna and they are used to retrieve the information stored in the transponder. Transceivers can be handheld, used to scan the body of the animals, and portable or stationary units in front of which the animals to be identified pass by. The data recorder stores and/or accumulates the information received by the transceiver from the transponder. Finally, the processing software transforms (processes) the information already recorded into easier-to-manage information (Hernandez-Jover, 2006; Espiñeira and Santaclara, 2016).

In animal ID, the information located into the transponder (information telegram) is translated into a 15-digit number, unique for each animal. This information is usually linked to other data of the animals (Hernandez-Jover, 2006; Espiñeira and Santaclara, 2016). The transponder is physically applied, injected or inserted in the animal to be identified. It can be located in different sites such as subscutaneously (e.g., ear base, neck, armpit or tail), in the intraperitoneal area or in the rumen, among others (Caja et al., 2004). When the transceiver approximates to the transponder, the latter is activated and the information in it is retrieved. Thus, the animal is identified, with all the additional information previously linked to the animal ID number (Rojas-Olivares, 2011).

In order to take full advantage of the RFID technology, the transmission frequency is of outmost importance. Low-frequency devices (134.2 kHz) are used for animal ID because they pass through water containing materials (i.e., body tissues) and are less susceptible to interferences at short distances (i.e., environmental and metal objects) than devices functioning at higher frequencies (Hernandez-Jover, 2006; Espiñeira and Santaclara, 2016). There are two different RFID technologies in use: full duplex (FDX-B) and half duplex (HDX) but all ISO readers must read both RFID technologies. In this work, both types FDX-B and HDX transponders were used.

In general, e-ID devices are easy and fast to read when correctly applied, with almost no human error while reading, but short distances between the animal and the readers are required. This is, in general, the most important concern related to animal ID and management (Caja et al., 2004, 2014). In practice, most commonly used RFID transponders are electronic ear tags, injectables and ruminal boluses. The two last are the ones of interest in this work.

Each ID system has its pros and cons (as seen in Table 1) regarding to reading efficiency, life span of the devices, management suitability, cost of the devices and animal welfare concerns. It is also important the compatibility of each system with the environment and living conditions of the animal (i.e., intensive, semi-extensive or extensive production systems in livestock, or freedom conditions in wild animals).

Injectables. They consist of encapsulated transponders that are directly injected in the animal. When correctly administered, they remain as a permanent ID during the life span of the animal. The transponder or microchip is encapsulated in a biocompatible, nonallergenic material that ideally promotes the growth of surrounding tissues for fixing the device in the specific area where they are applied. The locations may vary depending on the species. The most common and effective regions to apply injectable transponders in goats are subcutaneously in the groin, the armpit (named as **e-SUB** in the present study) and the metatarsal areas (Caja et al., 2004). The intraperitoneal (**e-IP**) region is also of interest (Caja et al., 2005) and enables the administration of large sized transponders at very early ages. Additionally, intramuscular application (**e-IM**) in the middle of the neck or between the scapuli is also used, especially in companion animals (Caja et al., 2014).

If correctly applied and registered, the injectables located in a very specific region are easy to read, either with handheld or stationary reader units. But there is the risk that the injected device migrates, making it difficult to locate or even to suffer breakage. In some cases, injectables could be lost after injection through the application wound before healing. In all these cases, they may become unreadable and therefore useless for animal ID (low or null readability). Moreover, due to migration, injected devices can be difficult to recover at slaughter (waste of time in the slaughterhouse) or be left in the carcass, with the risk of remaining in the food chain and of being consumed by humans (Caja et al., 2014; Espiñeira and Santaclara, 2016).

Rumen boluses. A ruminal bolus (**e-RB**) basically consists of a capsule of biocompatible and nonporous material, inside of which there is a transponder, as described by Caja et al. (1999). The boluses are orally administered to ruminants, who swallow the capsule, and are retained in the forestomachs, mainly in the reticulo-rumen, during the entire life of the animal. There are different types of e-RB, but those made of ceramics are the most commonly used, and of interest in this work. The boluses have a cylindrical shape and small diameter, which facilitates the oral administration, especially in young animals (Espiñeira and Santaclara, 2016). The e-RB will stay stable in the reticulum of the animal, not losing functionality through time when correctly administered. For reading the transponder, handheld reader devices need to be approached to the left side of the animal or, if stationary reading units are used, the antenna should be placed on the left side of the animal or pass through it. The e-RB are easy, efficient and safe ID devices, but when dimensions are not adequate, it may be difficult to administer or be easily lost by regurgitation or defecation. It has been seen that retention of e-RB depends on their dimensions and the specific gravity, which need to be optimized according to the age, weight and species (Ghirardi et al., 2006b; Carné et al., 2011; Caja et al., 2014).

Retention rate (RR) of e-RB has been studied in goats and sheep, cattle and camels. Some features such as material, weight and dimensions have been compared in order to clarify the retention law that explains their retention in ruminants. The explaining retention rate equations follow the logistic model in cattle, sheep and domestic goats as shown in Table 2:

$$y = \frac{A}{1 + b_0 \cdot e^{-(b_1 \cdot W + b_2 \cdot V)}}$$

where:

A = maximum value of bolus retention rate (usually, A = 100); b_0, b_1, b_2 = regression coefficients; *W* = weight; and *V* = volume.

Table 2. Mathematic models predicting the retention rate of ruminal boluses in different species.

Species	**Bolus retention rate (y, %)**	**Reference (year)**
Cattle	$y = \frac{100}{1 + 1{,}427 \cdot e^{(0{,}267 \cdot V - 0{,}160 \cdot W)}}$	Ghirardi et al. (2006a)
Sheep	$y = \frac{100}{1 + 1{,}139 \cdot e^{(0{,}763 \cdot V - 0{,}504 \cdot W)}}$	Ghirardi et al. (2006b)
Goat	$y = \frac{100}{1 + 0{,}734 \cdot e^{(0{,}787 \cdot V - 0{,}261 \cdot W)}}$	Carné et al. (2011)
Interspecies (sheep, SP = 0; cattle, SP = 5.8)	$y = \frac{100}{1 + 1{,}849 \cdot e^{(0{,}400 \cdot V - 0{,}429 \cdot W + SP)}}$	Caja et al. (2006)

Caja et al. (2006) suggested that it might exist a unique model to predict RR for all ruminant species from its dimensions, proposing a unique model for sheep and cattle which included a species coefficient (*SP*). No data of goats or camels was included in this equation because there was not available information at the date.

1.3.3. Other methods for natural ID

***Animal biometrics: muzzle print, retinal image and DNA molecular print*.** These methods use specific prints, marks or attributes of the animals (such as fingerprints in humans) to identify every individual animal.

The **muzzle print** was mainly done in cattle, dogs and horses and it consists in printing the external nose pattern of the animal, which is conformed with beads and ridges (like "islands" and "rivers"), using ink and paper like in the case of fingerprints. The muzzle print is unique for each animal and constant during its life. As a result, each specific pattern may be associated to a specific number and animal. Nowadays, the pattern is obtained using photography or computer vision and the digital images and numbers stored into electronic data bases (Petersen, 1922; Espiñeira and Santaclara, 2016; Kumar et al., 2018).

The **retinal image** is also unique for every specific animal (and human) and consists of the scanning of the blood vessel pattern on the retina which is a specific image of each animal for each eye. The retinal vascular pattern is a geometric configuration of the arterio-venous system, characterized by size, shape, concentration and length of veins and arteries. This pattern remains the same since birth and during the whole lifetime of the animal; it is created during fetal development. The obtained image can be checked by using a special software and associated with a unique ID number. For taking the image, the animal must be immobilized or retained in a head locker, but it is a very fast ID method (<1 min). It is a rapid, secure, reliable method of animal ID and able to be used for traceability (Rojas-Olivares, 2011; Awad, 2016; Espiñeira and Santaclara, 2016).

The **molecular markers** are based on DNA polymorphism analysis and permit to identify species, breeds and also individual animals. PCR (polymerase chain reaction) and its derivate methods such as STR (short tandem repeats), SNP (single nucleotide polymorphisms) and FINS (forensically informative nucleotide sequencing), are some examples. All these ID methods are, in general, expensive, but very accurate and highly reliable. They are the preferred authentication method in traceability. It can even be useful to identify a specific animal from portions of meat, preventing fraud. In this scenario, it can also be useful to identify and distinguish specific PDO (Protected Designation of Origin) products from others, which are susceptible of fraud **(**Caja et al., 2004; Espiñeira and Santaclara, 2016).

***Active-radiofrequency transponders*.** The transponders of this system incorporate a battery that provides extra power to send (transmit) information at specific times previously programmed. Thus, the recovery of the information is done automatically thanks to an antenna that receives the information encoded to each active-radiofrequency-identification (aRFID) device. The life span of the battery and the range of the emissions depend on the characteristics of both the transponder/transmitter and the receiver (Hernandez-Jover, 2006; Ruiz-Garcia et al., 2009; Espiñeira and Santaclara, 2016). In fact, there is a very wide range of aRFID device possibilities due to the recent advances on the technology used (Ruiz-Garcia et al., 2009). They are usually embodied in collars, leg bands, injectable, rumen boluses or even ear tags, depending on the animal and device features.

***Active biosensors for animal monitoring*.** These devices are active RFID transponders that, in addition, integrate biosensors for monitoring (gather information) of different variables of the animal, such as core temperature, rumen pH, position, activity, among others (Caja et al., 2016). They also need extra energy to work, but they are able to provide constant and specific information about each animal after being placed in different locations, such as: subcutaneous, intramuscular, intraperitoneal, ruminal, ear, leg, tail, vagina and others (Ruiz-Garcia et al., 2009; Caja et al., 2016; Espiñeira and Santaclara, 2016; Ellwood et al., 2017). The possibilities of these devices are widening nowadays due to the advances in technology (Ruiz-Garcia et al., 2009; Caja et al., 2016; Espiñeira and Santaclara, 2016; Ellwood et al., 2017).

The most common ID systems used nowadays and those of greater interest for this study, are visual tags (v-ID), because of the simplicity of the devices, and electronic devices based on the use of passive RFID transponders, as it is the case of injectables, located in different positions (intramuscular, e-IM; intraperitoneal, e-IP; and subcutaneous, e-SUB), and ruminal boluses (e-RB).

2. OBJECTIVES.

The specific objectives of this work are:

1. To compare the efficiency of distinct identification systems (visual tags; intramuscular, intraperitoneal and subcutaneous injectables; and electronic rumen boluses) in Iberian ibexes (*Capra pyrenaica*) from 2 different populations located in Spain, for a future use in population management and for the traceability and valorization of their game products.

2. To propose an interspecific and general model to predict the retention of rumen boluses which unify the preexisting models obtained in different domestic species (sheep and cattle) with the data available from domestic goat, camels and the here obtained from Iberian ibexes.

3. MATERIALS AND METHODS.

3.1. Animals and management.

3.1.1. Ethical permits for working with protected species.

Handling procedures for Iberian ibex were designed to minimize stress and health risks for subjects in accordance with the current guidelines for ethical use of animals in research and the European (2010/63/EU) and Spanish (R.D. 53/2013) legislations. This study complied with all Andalusian, Catalan, Spanish, and European legal requirements and guidelines regarding animal welfare, was approved by the Ethics on Animal Welfare Committee of the Universidad de Jaén and was authorized by the Dirección General de Producción Agrícola y Ganadera of the Consejería de Agricultura, Pesca y Medio Ambiente of the Junta de Andalucía.

All the handling procedures were the habitual for handling animals in the game reserves of this study.

3.1.2 Animals and populations.

The present work has been carried out in two different fenced stock reservoirs of Iberian ibexes sited within the natural distribution area of the species in Spain. Namely: 1) “El clot de l’Hospital” in the National Game Reserve of the Ports de Tortosa-Beseit -PTB- (Tarragona, Spain; 40º48’ N and 0º19’ E); and, 2) “El Toril” in the Sierra Nevada Natural Space -SNNS- (Granada, Spain; 37º03’N and 3º34’W).

Stock reservoir #1 (“El clot de l’Hospital” in the National Game Reserve of the Ports de Tortosa-Beseit, Tarragona, Spain; Fig. 2).

The PTB massif raises up to 1,441 m above sea level. The habitat is a mixture of mainly Mediterranean mixed forest of oaks (*Quercus* spp.), pines (*Pinus* spp.) and Mediterranean shrubs, combined with rocky areas.

Figure 2. Map of the NE of the Iberian Peninsula with the location (white dot in yellow circle) of the enclosure named “El clot de l’Hospital” in the National Game Reserve of Ports de Tortosa-Beseit, Tarragona, Spain.

The PTB enclosure (location #1) consists of 17 ha surface fenced with a double wire mesh, to avoid contact between captive and free-ranging ibexes and prevent disease contagion. There is also a quarantine area and a smaller fenced area connected to a corridor with a corral trap (50x100 m). Usually the fence and the corridor allow passing to another fenced area where feed and water were freely available. Ibexes were retained in the corral trap the day before handling. On the handling, they were driven through a funnel passage and finally trapped in a cage (1x1x1.5 m). Inside the cage, the animals were immobilized (4 legs tied) and blind-folded (cloth bag) previously to proceed to the health control and the ID of the animal.

In 2010 a total of 14 individuals were trapped and identified with subcutaneous injects (e-SUB) in the left armpit area and plastic ear tags. Retention data was recorded 2 and 4 years after injection. All the individuals were female.

Stock reservoir #2 ("El Toril" in the Sierra Nevada Natural Space, Granada, Spain; Fig. 3).

The SNNS includes Sierra Nevada National and Natural parks, covering a wide mountain massif with compact topography that goes from the southeast of Granada province to the western end of Almeria province and accounting for the highest peak of the Iberian Peninsula – Mulhacén, 3.479 m.a.s.l. –.

The SNNS enclosure (location #2) was built in 1993 to preserve the genetic diversity of Iberian ibex population of SNSS because of the sarcoptic mange outbreak few years before. It also has a double wire mesh fenced area of 30 ha surface with Mediterranean pine forest (*Pinus* spp.) and Mediterranean shrubs. Due to its dimensions and characteristics, there is a capacity of 60 individuals, but the herd size varies approximately 15% over a year.

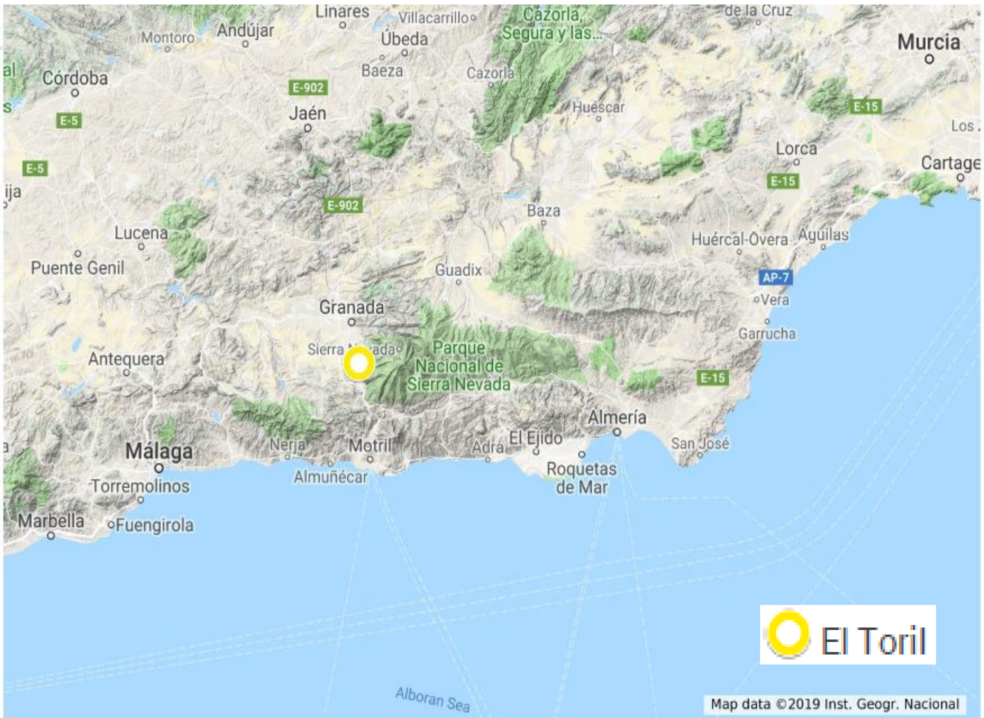

Figure 3. Map of the SW of the Iberian Peninsula with the location (white dot in yellow circle) of the enclosure named "El Toril" in the Sierra Nevada Natural Space, Granada, Spain.

The SNSS enclosure also has a corral trap (50x100 m), used to restrain the captive ibexes when necessary. With this aim, the animals are habituated to be occasionally handled by feeding them regularly in the corral trap. Similarly to location #1, the handling day they are retained in the corral trap and led through a funnel passage with a 1x1 m tunnel, where they are individually closed in a 1x1x1.5 m cage. For this study, once in the cage, the ibexes were immobilized and blind-folded in order to proceed with health monitoring and identification.

When a new individual was introduced to the enclosure, it was quarantined passing through a health control (i.e., blood sampling for diseases testing), identified (visual or electronic, depending on the year) and individual data recorded (i.e., weight, sex and estimated age). When health results came out, the healthy new individuals joined the rest of the herd. The herd of ibexes was permanently located in the enclosure and handled at least once a year (usually November) for health surveillance. They had free access to feed and water and reproduced naturally every year.

Captures started in 1993 applying v-ID from the start. After 2011, e-IM were also applied, completing the electronic ID of all animals with e-IP, e-SUB and e-RB from 2017 to 2019. The structure of the herd is shown in Table 3.

Table 3. Structure of the Iberian ibex herd used in the enclosure of the "El Toril".

Gender	Number of ibexes	Age		
		Mean	SD	Range
Males	28	4.50	2.19	0 to 7
Females	12	3.42	3.26	0 to 8
Unknown	4	-	-	-
Total	44			

3.2. Identification systems used.

3.2.1 Visual identification.

Ear tags. The ear tags used for v-ID were double flagged and made of polyurethane. They were inserted in the middle of the auricula using ear tag pliers when the animals were captured. In location #1 (PTB) ear tags were applied in both ears, large-sized (cattle size), always yellow and with the ID numbers written by hand for reading at long distances by using binoculars. In location #2 (SNNS) the v-ID systems consisted of a large variety of medium-sized ear tags (and occasionally collars instead of the ear tag), varying on models (sheep and goat ear tags), colors (yellow, green and blue) and printed codes of reference. During the annual captures, when a v-ID was lost, it was replaced by another v-ID, no matter the type (ear tag or collar), the color nor the dimensions.

Collars. Only in the SNNS collars were used occasionally. Enough space around the neck was allowed to avoid injuries to the animal. The colors used were yellow and green, and some had an orange plate. Never an animal with a collar was also wearing an ear tag.

3.2.2. Electronic identification.

Four different e-ID systems were used consisting of 3 types of injectables (e-IM, e-SUB and e-IP) and 1 type of rumen bolus (e-RB). Fig. 4 shows the specific location of the electronic devices used in this study and their features are summarized in Table 4.

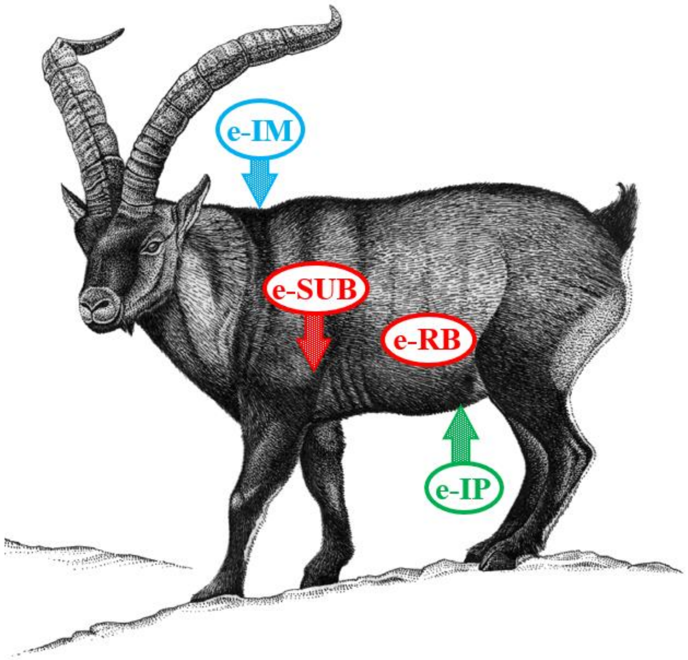

Figure 4. Location at the body of the animal of the different electronic ID systems used in the present study. **Arrows**: injectable transponders. **Blue**: injected intramuscularly within the scapuli (e-IM). **Red**: injected subcutaneously in the left armpit (e-SUB). **Green**: injected intraperitoneally (e-IP). **Red circle only**: rumen bolus transponder administered orally and retained in the reticle-rumen (e-RB). Image modified from https://www.uv.es/zoobot/.

Table 4. Characteristics of the different transponders used for the e-ID of Iberian ibex in this study.

Location	Transponder	Number	Brand	Product Code[1]	Technology	Length (mm)
#1 PTB	e-SUB	14	Texas Instruments	983 002	HDX[2]	32
#2 SNNS	e-IM	4	Insvet	938 001	FDX-B[3]	12.2
		43	Datamars Iberica	953 009	FDX-B[3]	12
	e-SUB	44	Datamars Iberica	981 007	FDX-B[3]	32
	e-IP	44	Datamars Iberica	981 007	FDX-B[3]	32
	e-RB	30	Datamars Iberica	964 003	HDX[2]	32
Total	All types	180	-	-	-	-

[1]According to ICAR: https://www.service-icar.com/tables/Tabella3.php; [2]Half Duplex, alternative communication; [3]Full Duplex B, simultaneous communication.

Intramuscular injects. They were the original e-ID used in location #2 (SNNS) from 2011 where 44 ibexes were injected with 47 **e-IM** (4 animals were injected with 2 microchips because the first was not detected at health monitoring). The devices (small transponders or microchips similar to those used for companion animals) consisted of 4 Insvet Microplus (Esplus, Huesca, Spain) FDX-B 2x12.2 mm treated with the polymer Parylene for antimigration and 44 Datamars Iberica (Albacete, Spain) FDX-B 11 mm glass encapsulated transponders, as summarized in Table 4. All e-IM devices were applied intramuscularly by trained operators between the scapuli, without anesthesia or previous disinfection and using single shot sterilized injectors with disposable needles (25x2.5 mm) provided by each manufacturer. No e-IM transponders were used in location #1 (PTB).

Subcutaneous. The **e-SUB** devices were initially tested in location #1 (PTB) from 2010 to 2014 and in location #2 (SNNS) from 2017 and consisted of 14 Texas Instruments 32 mm transponders and 58 Datamars Ibérica FDX-B 32 mm transponders, respectively. They were subcutaneously injected in the left armpit using a Texas multi-shot injector with cartridges of 10 and immersed in an iodine gel solution (Betadine oplossing, Dagra, The Netherlands), according to Conill et al. (2000, 2002). Injection body sites were also disinfected before each injection with another iodine solution (Braunol, B. Braun Medical, Jaén, Spain), which was also used to clean the needle after each injection. All operators were trained before injection.

Intraperitoneal injects. The **e-IP** devices were only used in location #2 (SNNS) from 2017 and consisted of 44 Datamars Ibérica FDX-B 32 mm. They were injected in the intraperitoneal region, on the right side of the ventral midline (*linea alba*) and above of the navel, according to the methodology proposed by Caja et al. (2005) in piglets and using a Texas multi-shot injector with interchangeable needles that were immersed in an iodine solution (Betadine, Braunol, B. Braun Medical, Jaén, Spain) before each injection. The specific region of application was 4 cm laterally to the *linea alba*, and 10 cm caudally from the navel. This location was chosen in order not to interfere with the other left-sided applied transponders, and because injection was easier when the devices were applied on the right part of the body being the goats immobilized on their back (head on the left of the operator). After the needle traversed the abdominal wall, the injection direction changed to perpendicular and the transponder was released. Injection sites were also disinfected before each injection as above indicated. All the operators were trained before injection.

Rumen boluses. The **e-RB** devices were only used in location #2 (SNNS) from 2017 and consisted of 30 cylindrical ceramic rumen boluses (20x66.6 mm length, 71.4 g weight) equipped with Texas HDX of 32 mm transponders. They were orally administered using appropriate balling guns, as previously described by Caja et al. (1999) and Carné et al. (2009).

The weights at application ranged between 28 and 45 kg BW. For the lower weights, the e-RB were applied directly by hand, using leather gloves for operator's finger protection.

3.3. Reading of the different identification systems.

The restrained ibexes were read using the same handling procedure at each health control in both PTB and SNNS locations. Ibexes in the enclosure were captured as previously described and immobilized in order to check for the 5 different identification systems. A previous list of all the animals in the stock reservoir and their ID numbers was available and the handheld animals and ID devices read recorded. Information on "absent" animals (not captured / restrained, dead or escaped) and not readable ID devices (lost, broken or failed) were also taken.

3.3.1. Visual reading.

All v-ID ear tags were checked in order to identify the animal. If the v-ID tag was missing or illegible, the available e-ID was used to retrieve the animal. The identification and recording procedure were made by visual identification when the animals were restrained. Data was recorded by hand and then transferred to an electronic support.

3.3.2 Radiofrequency reading.

All e-ID devices were also read before and immediately after application to check their functioning, to ensure the correct application and localization post-application (especially important in e-RB) and at annual health controls. In location #1 (PTB), used to test the possibility of using e-ID in Iberian ibex, the devices (e-SUB) were applied in 2010 and read only after 2 and 4 years (2012 and 2014). In location #2 (SNNS), the e-IM were routinely applied to reinforce the v-ID data during a 6-year-period (since November 2011 until November 2017) before the current study started. Nevertheless, in this case no data on early losses (d 1 to 3 after application) or failures and breakages were recorded before November 2017. See Table 5 and Figure 5.

Readability of all e-ID devices was performed using Datamars Ges3 and Felixcan full-ISO handheld transceivers able to read ISO RFID transponders of half-duplex (HDX) and full-duplex B (FDX-B) technologies at a maximum distance of 20 to 30 cm for the 32 mm transponders, as established by European Commission Regulations EC 21/2004 (EC, 2004) and EC 933/2008 (EC, 2008b) on this issue. The restrained animals were scanned from both sides with the readers to detect each e-ID type and their presence or absence recorded at each control.

Table 5. Mean time elapsed after application of the different e-ID systems in Iberian ibexes.

ID type	Application dates	Readings (elapsed time, d)[1]				Study length	
		R1	R2	R3	R4	Days	Years
e-IM	23/11/11 to 9/11/17	721	899	1025	1346	1867	5.0
e-SUB	7-9/11/17	3	137	371	580	580	1.6
e-IP	7-9/11/17	3	137	371	580	580	1.6
e-RB	7-9/11/17	3	137	371	580	580	1.6

[1]Mean of time elapsed between application dates and reading events (R_i; i = 1 to 4).

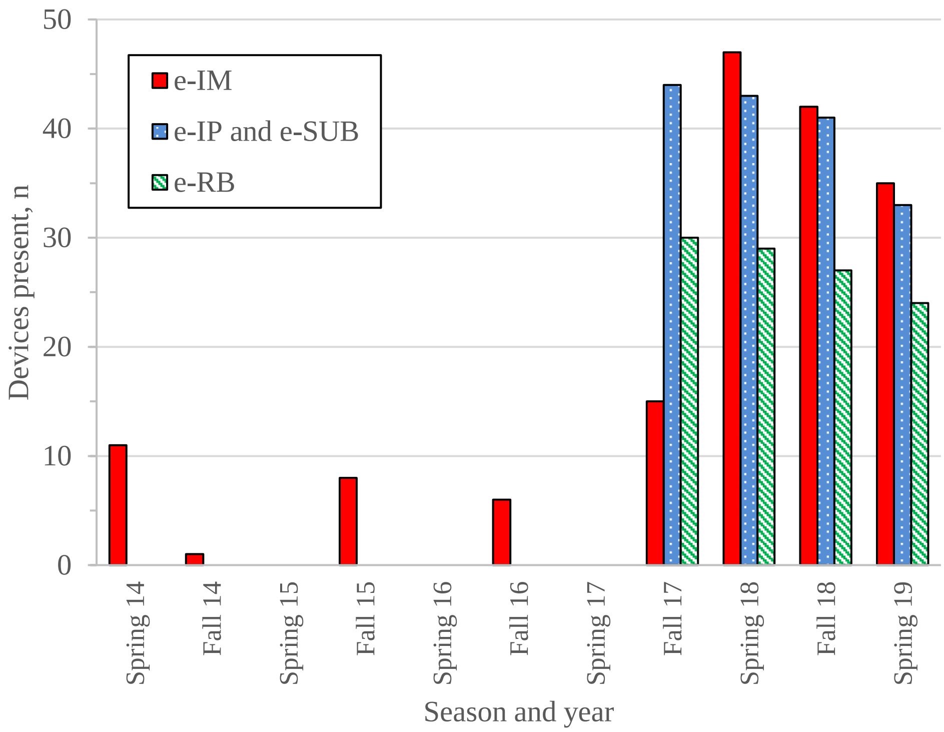

Figure 5. Number of e-ID devices present in Iberian ibex during the study grouped by application and reading seasons from 2014 to 2019.

3.4. Statistical analysis.

3.4.1 Variable standardization and recording.

When an e-ID device was not detected during a reading event but it was detected at the next reading event, the electronic device was marked as "unreadable" at that particular event. These values were used for calculating the reading efficiency but not for retention rate. Retention rate used data from readable devices at the last reading in which the animal was present. Data of absent or dead animals was also considered when available. For animals with two e-IM devices, each ID device applied was considered as a different individual. No data from Iberian ibexes with more than 5 yr of e-ID devices applied were used because of the low number of individuals available (6 individuals at year 5.5, and 2 individuals at year 7.5).

Reading efficiency (RE) or readability (%) was calculated as the number of "read devices" with regard to the "animals captured" less "lost", "not read" and "unread" devices, being:

$$RE\ (\%) = \frac{n\ read\ devices}{n\ animals\ captured}\ x\ 100$$

Retention rate (RR) (%) was calculated considering the number of potentially "readable devices" all the read devices and those devices that were read again in posterior events, being:

$$RR\ (\%) = \frac{n\ readable\ devices}{n\ animals\ captured}\ x\ 100$$

The yearly losses were calculated considering the time of the study in years (*Y*) as:

$$R_Y = (RR/100)^{1/Y},$$

And, yearly RR was calculated as:

$$Yearly\ RR = 100 - R_Y$$

Resulting the predicted values of RR after x years (*xY*) as follows:

$$RR_{xY} = (RR_{xY}/100)^{xY},$$

3.4.2. Statistical models and procedures.

The evaluation of different ID systems was conducted under a randomized incomplete block design using a total of 58 individuals of *Capra pyrenaica* of different ages and habitat conditions within the 2 locations mentioned.

Readability and retention rates of ID devices were analyzed with the CATMOD procedure of SAS (version 9.4; SAS Inst. Inc., Cary, NC) on the basis of the categorical nature of these variables. The procedure PHREG (proportional hazard regression) for survival data of SAS was considered preferable to the logit model, as indicated by Caja et al. (2014) aiming to avoid the possible bias produced by the different number of animals monitored until the end of the study in addition to introducing the effects of the location sites and ID devices. Furthermore, the readability data were compared on a yearly basis according to Seroussi et al. (2011). When no losses of devices were observed, they were simulated (1 to 3) to allow running the statistical analysis. The differences between means were declared significant at $P < 0.05$, unless otherwise indicated.

Interspecies bolus retention rate model was calculated by means of a nonlinear least squares regression model, using the "minpack.lm" package (Timur et al., 2016) of R software v.3.5.2 (R Core Team, 2018), assuming a logistic distribution, as indicated for bolus retention rate in cattle and sheep (Caja et al., 2006). The model included the weight (W) and volume (V) of the boluses and the animal species (SP) as independent covariates, being:

$$y = \frac{A}{1 + b0 \cdot e^{(b1 \cdot V + b2 \cdot W + SP)}}$$

Where: y = bolus retention rate; b0, b1, and b2 = the regression coefficients; and, A = the maximum value of bolus retention rate.

The structure of the data used from different bolus types and ruminant species were a total of 63 data from 5 species (sheep = 13, goat = 20, cattle = 19, camel = 8, ibex = 3) corresponding to 5,644 animals (sheep = 1,593; goat = 2,265; cattle = 1,196; camel = 556; ibex = 33).

4. RESULTS AND DISCUSSION.

4.1. Location #1 "El Clot de l'Hospital"

In 2010, a total of 14 ibexes were identified in the left armpit in the PTB enclosure. Two and four years later (2012 and 2014), the 100% of the individuals captured were correctly identified by using the e-SUB. In 2014 only 13 individuals were captured, but all retained the e-SUB devices. No infections or apparent injuries were reported in the injection site. Thus, for the 4-year study length considered, both readability and retention rate of the e-SUB were of 100% (see Table 5).

4.2. Location #2 "El Toril"

A total of 44 wild goats were identified during the study length in the SNNS enclosure using 5 different ID systems (i.e., v-ID, e-IM, e-SUB, e-IP and e-RB). The total number of ID devices applied and their long-term performance at all locations are summarized in Table 5.

From the 44 captured and identified animals at the beginning of the study in "El Toril", 3 animals died (two after the 2nd reading -R2- and another one after the 3rd reading -R3-). Moreover, 8 more animals were absent during the last reading, one of which was also absent from the 1st reading (R1).

Table 5. Actual performances of the distinct ID systems used in Iberian ibex and predicted retention rate after 10 yr of application

		Devices, n			Years		Retention	Yearly mean		Predicted after 10 yr	
Location	ID type	Applied	Present animals[1]	No readable	of study	Readability, %[2]	at the end of study, %	Retention, %[3]	Losses, %[4]	Retention, %[5]	Losses, %[6]
PTB	e-SUB	14	13	0	4.00	100	100	100	0	100	0
SNNS	e-IM	47	17	1	5.00	95.82[b]	94.12[c]	98.8[ab]	1.2	88.6[b]	11.4
	e-SUB	44	33	2	1.59	96.46[b]	93.94[c]	96.1[c]	3.9	67.5[d]	32.5
	e-IP	44	33	1	1.59	97.98[ab]	96.88[b]	98.0[b]	2.0	81.9[c]	18.1
	e-RB	30	24	0	1.59	98.57[a]	100[a]	100[a]	0	100[a]	0

[1] Recaptured at the end of the study

[2] Calculated readability rate as the mean of the readability of every reading event. Readability of event = (n read devices/n captured goats) x 100.

[3] Estimated as yearly retention rate (RR): $R_Y = (RR/100)^{1/Y} \times 100$; y = duration of the study in years.

[4] Yearly losses: RR = 100 – R_Y.

[5] Estimated values of RR after 10 years, using the R_Y previously calculated: $R_{Y10} = (R_Y/100)^{10} \times 100$

[6] Estimated values of losses after 10 years, using the corresponding values of R_Y previously calculated: $100 - R_{Y10}$.

4.2.1. Ear tags and collars.

More than the half of the animals (undetermined) under study did not wear v-ID at the annual capture, but the e-ID data reported previously applied ear tags. In these cases, new v-ID (mainly ear tags) were applied to re-identify the animals. This fact indicated that their ear tags were lost between two consecutive readings. Collars showed better retention than ear tags, although no objective data supported this assumption.

Obtained results agreed with the results reported on v-ID systems in domestic goats (Caja et al., 2014). So, despite being cheap, easy to apply and to read, they are not a recommended method for Iberian ibex ID as a consequence of the huge annual losses.

4.2.2. Intramuscular.

The e-IM were read within a large area of the animal's body covering from the scapuli to the neck. Whether this was due to migration of small transponders (despite having an antimigratory treatment that seems to be ineffective) or to the original place of injection, it is unknown. Reading difficulties were also detected, being necessary repeated scanning for a positive reading. Reading efficiency of the e-IM varied markedly at the different reading events (Fig. 6).

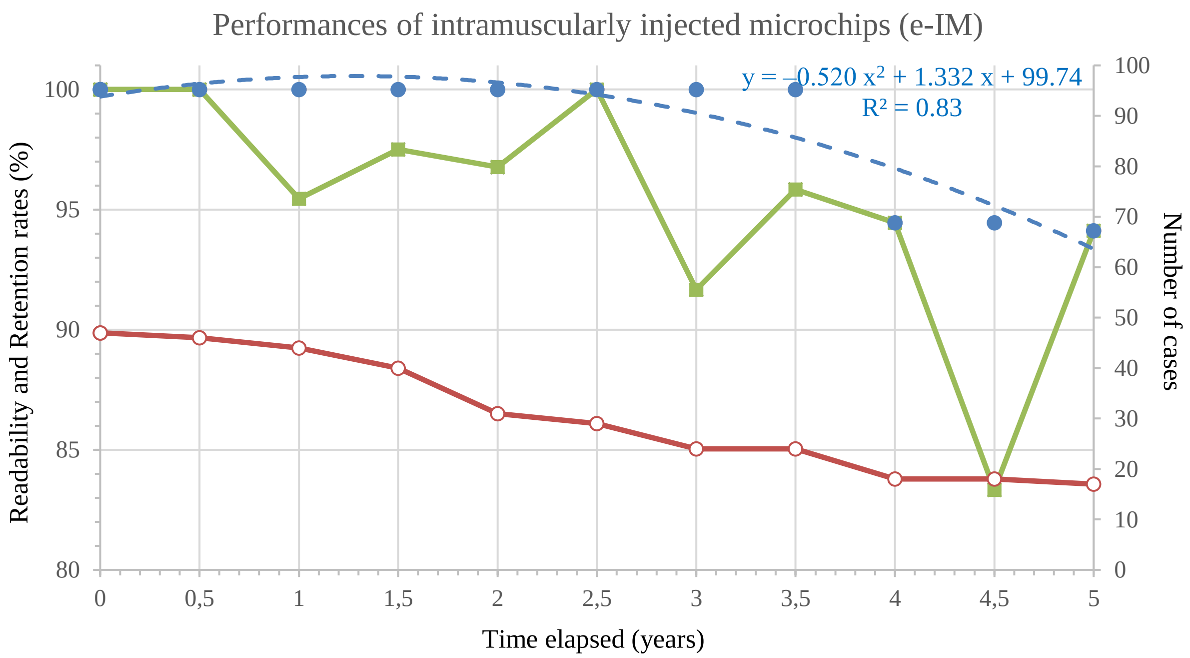

Figure 6. Number of cases (open red circles), readability (green squares) and retention rate (blue closed circles) of the microchips of 12 and 12.2 mm injected intramuscularlly between the scapuli (e-IM) of Iberian ibexes. A logarithmic tendency was observed for retention rate (R^2 = 0.83).

At the first capture of the study (November 2017), it was not possible to detect a total of 6 e-IM previously injected so, assuming that they were lost, new microchips were injected by the operators. However, during the following reading events, some of the first undetectable microchips were detected again. That shows the difficulty to find the small sized microchips (11-12.2 mm) in the body of the animals as a consequence of migration and of the small reading area of their RFID components (i.e., small antenna). Moreover, in many cases, the e-SUB was detected when checking for e-IM, the reader being placed in the cranial part of the body of the animal. Nevertheless, under future use conditions this problem will not be a concern because only 1 type of e-ID will be used in each animal.

The variable readability observed may also be a consequence of the different operators carrying out the reading procedure which was made more difficult by the unexpected location of the transponder and the large area of possible migration. It should be also stressed, as previously mentioned, that e-IM had less reading distance than the other transponders used, and its reading collided with that of the e-SUB of 32 mm.

Retention rate was constant for the first 3.5 yr of the study and losses were only detected from the 4 yr and thereafter. It should be stressed that the drop in retention at the end of the study corresponds to only 1 lost e-IM (i.e., 1 device lost during years 4-5 represents more than 5% decrease of retention). The rest of e-IM applied at the beginning of the study were still detectable when the animals were captured at the R4.

Despite the low reading efficiency, predicted retention rate after 10 years was moderately high (88.6%) although lower than the official data approved for livestock species (RR > 98%). One concern related to e-IM is the fact that since they are applied intramuscularly, and their size is small, predators and scavengers could swallow the e-ID devices with the meat of the animal. Moreover, if the animal is hunted, the undetected e-IM could be a risk for consumers.

4.2.3. Intraperitoneal.

Most e-IP transponders were located on the injection area, indicating a very low migration (no data recorded). One e-IP was initially undetectable at R2 and during the following captures and readings. Since the loss was detected at the 3rd day of the study, it may indicate that the transponder was most probably injected in the lumen of the intestines and lost with feces.

Readability rates of e-IP were few variable and high, greater than 95% in all reading events (Figure 7). The observed variations were considered to be effect of the human factor: lack of experience and short reading time, among others.

Retention rate of e-IP also showed the initial loss of one device after the first reading (R1), but it steadied for the rest of the study. The rest of e-IP applied were still detectable and located in the injection area at the end of the study (Table 5 and Figure 7).

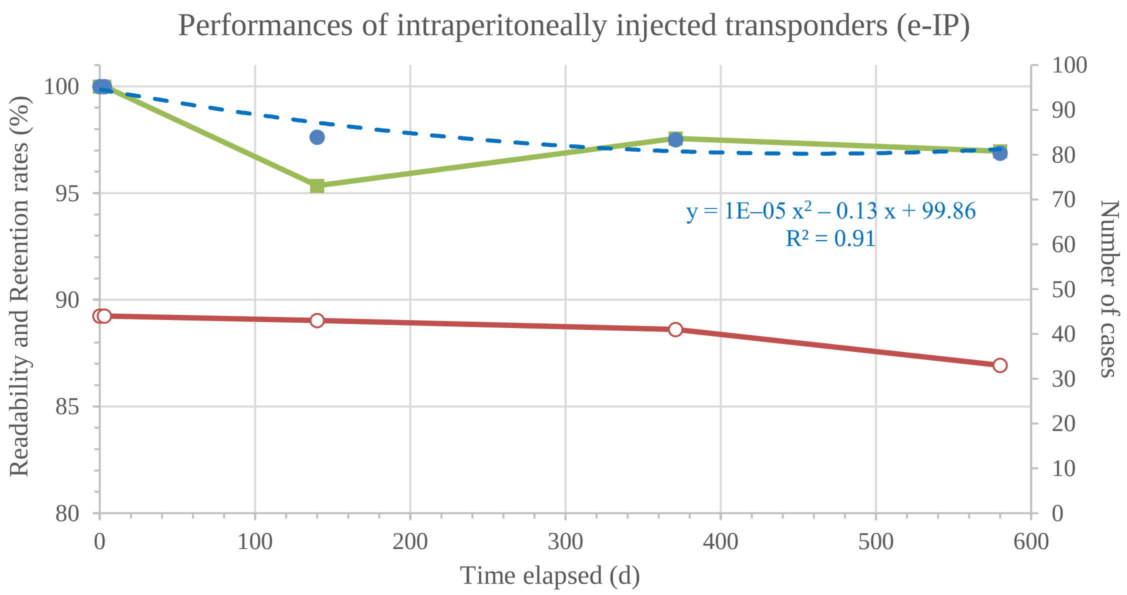

Figure 7. Number of cases (open red circles), readability (green squares) and retention rate (blue closed circles) of the intraperitoneally injected transponders (e-IP) of 32 mm in Iberian ibexes. A logarithmic tendency was observed for retention rate ($R^2 = 0.91$).

In our knowledge, there are few data available on the use of e-IP in the long-term in goats. However, Pinna et al. (2005) found 100% readability of the transponders after 28 d of injection in new born kids (d 1 to 5 of age); no early losses or casualties were found in this case. This suggests that a correct injection may avoid early losses, achieving 100% readability and retention. Consequently, e-IP were considered, as previously suggested, a very safe and reliable ID system.

Predicted retention rate of e-IP after 10 yr was 81.9% (Table 5), lower than observed for e-IM. Despite this low RR values, these devices are of interest because they can be applied at early ages, without later losses nor compromising the health or even the wild natural life of the young animals. Moreover, the observed losses seem to be related to misapplication of the device, which means that with more experience, these losses could be even smaller. Since the e-IP devices stayed in the abdominal cavity, among the intestines, this system will be safe for humans consuming game products. On the contrary, the transponder may be consumed by predators that usually feed on viscera (i.e., vulture, fox, wolf…).

These factors may be a key for taking the final decision about the most convenient e-ID system to implement in wild goat populations of Iberian ibexes.

4.2.4. Subcutaneous.

Reading of the e-SUB devices injected in the left armpit was easy and fast, as expected because of their size (32 mm), but showed them in a large area (not recorded data). The reading area was extended from the neck, to the scapulae or the breast of the animal. This may have been consequence of migration or diverse application points. The armpit is one of the regions with lower tissue reaction after injection of transponders, which finally means greater migration (Caja et al., 2014). This fact agrees the large area where the devices were read.

Despite these migration concerns, reading efficiency was high (>93%) and only 3 reading failures occurred during the study (Table 5 and Figure 8).

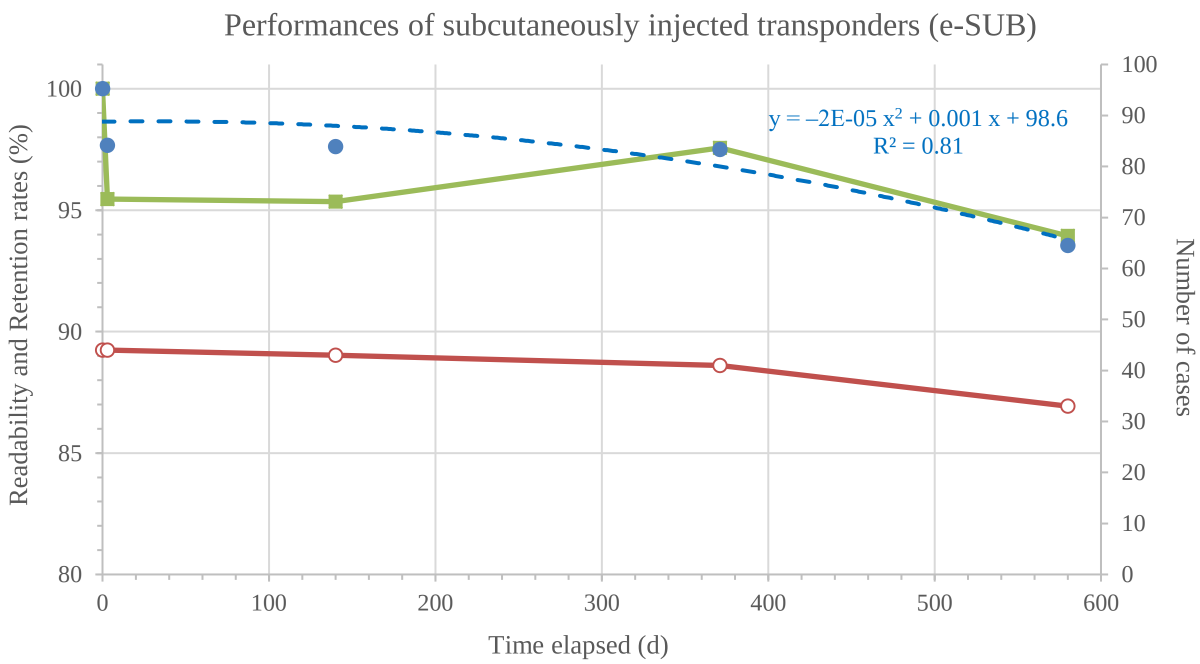

Figure 8. Number of cases (open red circles), readability (green squares) and retention rate (blue closed circles) of the subcutaneously injected transponders (e-SUB) of 32 mm in the armpit of Iberian ibexes. A logarithmic tendency was observed for retention rate (R^2 = 0.81).

Immediately after injection it was detected one loss of e-SUB (1 device, representing approximately 2%), that was a consequence of expulsion from the injection site. Most probably the e-SUB stayed in the channel made by the needle after application, from where it got out due to the strong movements of the armpit when the animal was released. No suture or fast-acting adhesive (i.e., cyanoacrylate) was used for blocking the needle hole until the formation of scar tissue. Nevertheless, these losses after injection of e-SUB were not observed in location #1 (PTB), where few animals were injected at each time and their processing speed was very low. In this case, scar formation may have been enough for retaining the transponder. Consequently, greater experience of the operators, low speed of processing at injection or the use of adhesive could reduce these human-factor caused losses.

Moreover, e-SUB injection in the armpit was more difficult than in other areas because its mobility, due to the proximity of the joint, and because of the need of placing the needle parallel to the body, inside a pinch of skin and between the fingers of the operator.

According to the obtained results and under the conditions of this study, the armpit region was the less effective injection area compared to the other application locations but, apart from the injectable lost right after application, considered as an early loss, there was only another injectable unreadable at the last reading event (R4). The rest of the injected e-SUB were still readable at the end of the study. Thus, if early losses were avoided, this e-ID system could be also suitable for Iberian ibexes readability and retention rate could be obtained as earlier discussed in location #1 (PTB). Agreeing this, predicted retention rates after 10 yr were low in SNNS but maximal in PTB (Table 5).

Regarding to predators and scavengers, the e-SUB devices could be easily ingested due to their location. Nevertheless, as indicated by Caja et al. (2014) the 32 mm e-SUB (armpit location) are a useful tool for tracing caprine carcasses in the slaughtering line for experimental purposes. Thus, it could be an interesting device to be used for the traceability of game products.

High values of readability and retention rates (98 to 100%) have been reported for e-SUB in the armpit of domestic goats in the long-term (3 yr; Caja et al., 2014). These values are slightly higher than those obtained in the present work. Further long-term studies should be done in order to determine whether e-SUB perform better in domestic goats than in Iberian ibexes.

4.2.5. Rumen bolus.

Values of readability and retention rate of e-RB were very high (96 to 100%) throughout all the study (Table 5 and Figure 9) and migration, because of their effective retention in the reticulum-rumen, was not detected. Fast and easy reading, with low reading failures were recorded. Only 2 misreading (R1 and R2) of 1 device were detected among the 140 total reading procedures carried out during the experiment.

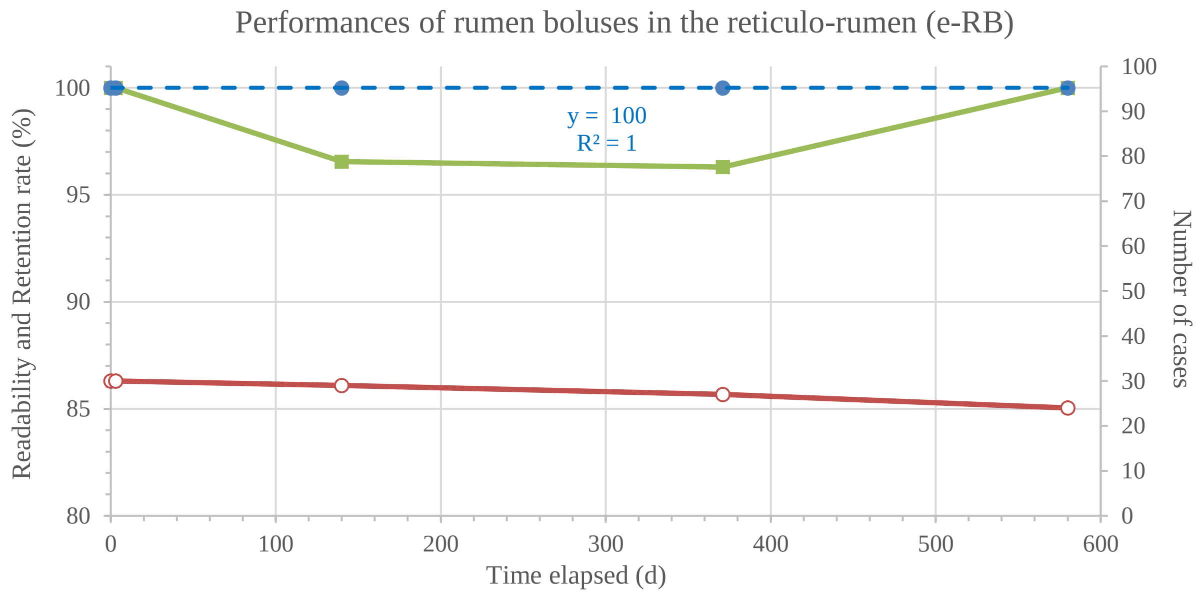

Figure 9. Number of cases (open red circles), readability (green squares) and retention rate (blue closed circles) of rumen boluses (e-SUB) with transponders of 32 mm in the reticulo-rumen of Iberian ibexes. A flat tendency was observed for retention rate ($R^2 = 1$).

All e-RB were read at the last reading event (R4) indicating a maximum retention rate (100%) throughout the whole study. No losses, failures or breakages of the devices were reported. It should be stressed that given the lower total number of e-RB administered (Table 5), when compared to other e-ID systems used, the impact of the occasional readability failures (1 individual) was greater when compared to other devices.

This system proved to be safe when the boluses were administered by hand to young individuals (28 kg body weight) and to have a full retention rate, independently of the size of the animals involved in the study (28 to 45 kg). The full retention of the e-RB used in the Iberian ibexes was unexpected and disagree with the results obtained in domestic goats, which are able to regurgitate the standard boluses used as reported by Caja, et al. (2014) in Spanish, Portuguese and Italian domestic goats. In domestic goats, retention rates of e-RB with specific gravities above 3.3, averaged 96.8 ± 0.8%. Losses depended on the breed and ranged from 0.2 to 8.9%. Nevertheless, in other studies, losses were even greater (2.0-10.3%), depending on the breed and environmental conditions (i.e., free-range herds obtained grater losses). Breeds under extensive conditions seemed to suffer from greater losses in some studies but lower losses in others.

In the case of Spanish domestic goats, retention rates averaged 98% (Caja, et al., 2014), even reaching 99.6% in dairy goats under extensive conditions. In the USA values of 100% have been reported under extensive conditions and 97.8% under semi-intensive conditions (Caja et al., 2014).

Some authors suggested that behavioral and diet differences could negatively affect the retention of e-RB in goats. Jumping and fighting, as well as high concentrate diets could increase the losses (Capote et al., 2005; Carné et al., 2009, 2011).

The Iberian ibexes of location #2 (SNSS) of the present study were kept under semi-extensive conditions, supplemented with hay available *ad libitum*. No concentrate was given to those individuals. Behavior of Iberian ibexes (*C. pyrenaica*) is supposed to be wilder and more aggressive than domestic goats (*C. hircus*) and, consequently, greater losses were expected in Iberian ibexes during the present study. It had been hypothesized that some goats could develop the ability to regurgitate some hard components of diet, according to the environmental conditions; nevertheless, it was not the case of the individuals in the present study because no losses (i.e., regurgitation or defecation) were observed. More research should be done in order to identify the causes for which the retention of e-RB is so high in Iberian ibexes. Estimated RR calculated after 10 years was 100%, higher than the value obtained by Carné et al. (2008) for heavier boluses.

An additional advantage of e-RB is that they are also safe to predators and scavengers, the bolus intake being rejected and so being able to be found in the ground among the bones and remains of the dead animals. Moreover, e-RB is a safe system for human consumption, being the applied devices easily retrieved at the slaughterhouse or removed on-field from the game products.

The obtained results, the safety and easiness of the administration by trained operators, professionals, and the good performances when applied to wild or free-range animals makes this system suitable for Iberian ibexes and one of the best in the present work.

Electronic rumen boluses interspecific retention law.

The obtained results of the retention rate of boluses were added to those previously published for cattle (Ghirardi et al., 2006a), sheep (Ghirardi et al., 2006b), goats (Carné et al., 2011) and dromedary camels (Caja et al., 2014) to obtain a multispecies retention law in the reticulo-rumen, as previously proposed by Caja et al. (2006). The collected data, corresponding to 66 data, including those of Iberian ibexes, is shown in Figure 10.

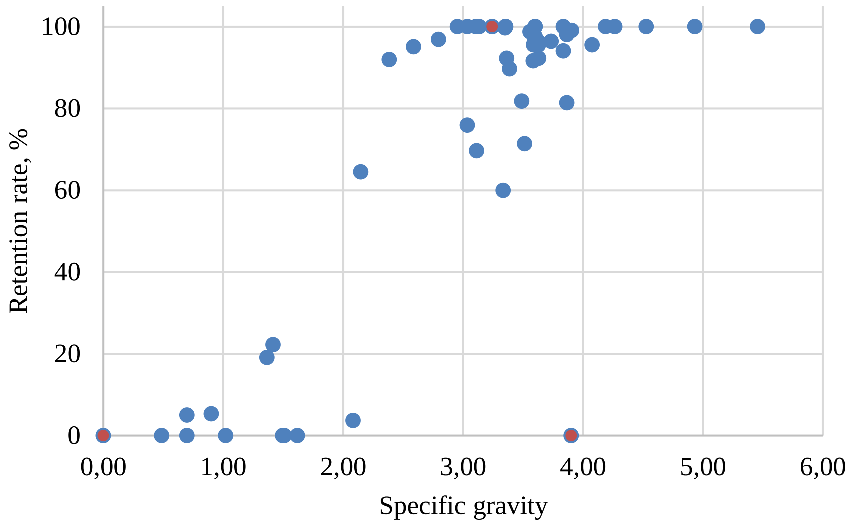

Figure 10. Retention rate of rumen boluses (e-RB), containing transponders of 32 mm in the reticulo-rumen, of different ruminant species according to their specific gravity (SG = weight/volume). Values of Iberian ibexes are marked in red.

The interspecific equation for the retention rate of the bolus being the following ($R^2 = 0.96$; RSE = ±8.99 and $P < 0.001$):

$$y = \frac{100}{1 + 0.490 \cdot e^{1.601 \cdot V - 0.796 \cdot W + SP}}$$

Where the species coefficients (SP) obtained were:

Sheep = 1.576

Goat = 3.721

Cattle = 5.667

Camel = 4.874

Ibex = 16.612

Predicted values for Ibex will improve if more data from further research is incorporated to the regression but, compared to other species, they will require the heaviest e-RB among the species used. Interspecific retention rate law is shown in Figure 11, as calculated for the usual specific gravity of ceramic boluses (SG = 3.3).

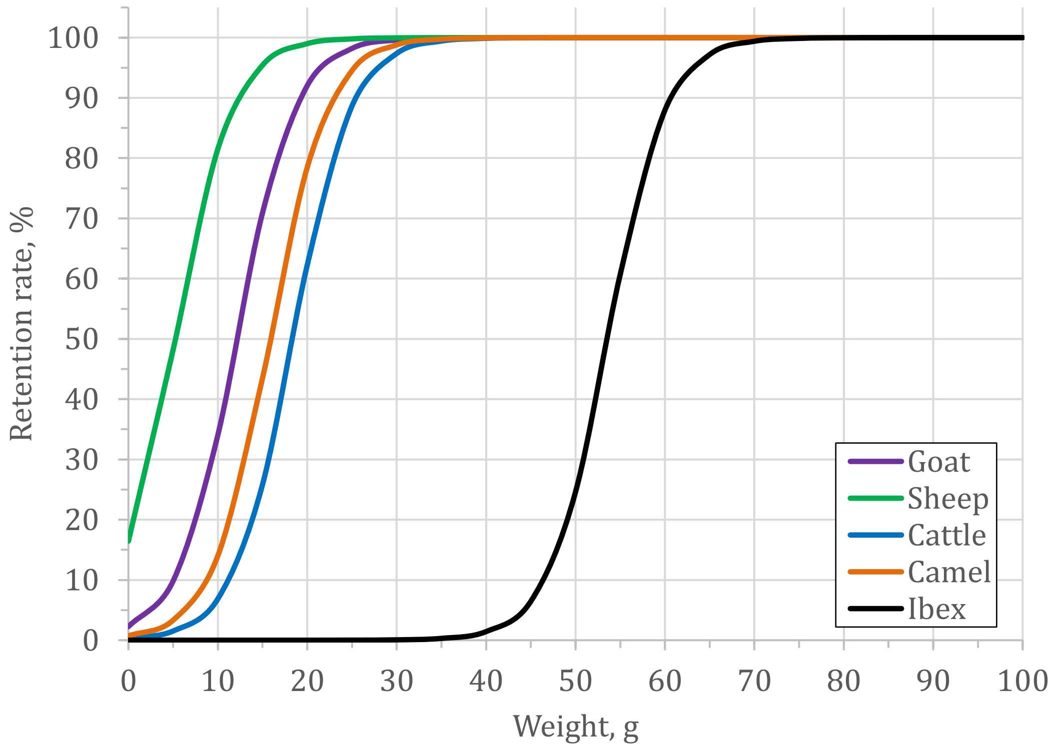

Figure 11. Predicted retention rate of rumen boluses (e-RB), containing transponders of 32 mm in the reticulo-rumen and a common specific gravity (SG = 3.3), of different ruminant species according to their dimensions.

5. CONCLUSIONS.

The obtained results allowed us to draw out the following conclusions for the identification and traceability of Iberian ibexes:

- Visual ear tags showed the lowest retention rate and are not recommended. If necessary, small ear tags or the use of notching would be preferable. No problems were reported when collars were used in adult animals.
- The greatest actual and predicted values of readability and retention rates were obtained with the electronic devices e-RB and e-IP, both using 32 mm transponders. No application problems were found when applied in individuals greater than 28 kg body weight.
- Despite the expected differences between domestic and wild goats, no bolus losses were detected in Iberian ibexes.

- Other injectable electronic devices, such as e-IM and e-SUB, showed lower and variable values of readability and retention rates due to losses after application and migration in the body of the animals. Values could improve with greater training of the operators and if larger transponders were used (32 mm instead 12 mm).
- Regarding safety for predators, scavengers and game products consumers, the e-RB were also preferable.
- Finally, according to these results, the e-RB seems to be the most recommendable device for the ID of Iberian ibexes in practice.

6. IMPLICATIONS.

6.1. Implementation proposal: configuration of an electronic reading system for *Capra pyrenaica*.

There is evidence that e-ID was preferable to v-ID and that the combination of device, transponder technology and reading strategy may allow for a more efficient system in practice. It has been reported that transponders of HDX technology used alone give the best reading efficiencies with vertical left-sided antennas placed in narrow alleys (Caja et al., 2014).

The use of e-RB with 32 mm transponders of HDX technology, administered after capture to ibexes greater than 30 kg while restrained in a net bag, is foreseen as the most efficient ID system. Afterwards, the use of large frame antennas (i.e., 90 cm) on the left side of tight (i.e., 40 cm) one-way alleys may allow full dynamic reading. The installation of the antenna in the end of the alley should be avoided to reduce the speed of the animals (i.e., curved or angled alleys and with steps are preferable).

Several antennas could be installed in strategic points where the population of Iberian ibexes to be controlled pass through (i.e., watering, salting and feeding points) and where automatic reading and transmission could be achieved with the help of batteries or solar panels as energy source.

In the future the use of aRFID could also be of interest and could have incorporated GPS and / or temperature biosensors, in order to locate the animals or to know if the animal is alive or the device was lost (e.g., low temperature and stationary position). Batteries are the hugest concern of these devices and large antennas are also necessary to retrieve the information from long distances. Active RFID has not been contemplated in the present work and further studies are necessary before using aRFID in Iberian ibexes.

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