

## Plastinated macroparasites, an alternative resource for use in practical lessons

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### Summary

The plastination technique is a recently developed option to preserve biological tissues widely used in human and veterinary anatomy. This process allows one to obtain real, safe, clean, dry, resistant and permanently stable material without any additional treatment or maintenance. All these advantages are associated with the rapid expansion of plastination during recent decades in a high number of educational institutions. However, the studies of parasite conservation through plastination are scarce, although the material obtained has a high quality, as prior references have shown. In this study, 141 students of Veterinary Medicine and Pharmacy University Degrees used, in a blinded study, plastinated specimens (experimental group) or wet specimens (control group) during three consecutive practical sessions. Afterwards all the students completed anonymous questionnaires on both knowledge and satisfaction. The results demonstrated that

plastinated parasites are a valid and satisfactory alternative to be used as a teaching/learning tool during practical lessons. Moreover, with regard to the knowledge of morphological structures, the results from the questionnaire of assimilated knowledge showed no differences between the use of plastinated and formaldehyde-preserved parasites.

### **Keywords**

Innovation – Learning tool – Parasite – Plastination – S10 technique.

### **Introduction**

The plastination technique (1) is the most recent and important option for the conservation of biological material, as shown by its rapid expansion in recent years (2). During plastination, body fluids and part of the adipose tissue are slowly replaced by polymers under vacuum conditions (1). In this way, real biological preparations are obtained with advantageous characteristics when compared with traditional conservation. Plastinated samples are clean, dry, resistant, durable and can be handled with no safety precautions such as gloves or masks. In addition, they require no special treatment or storage conditions (3). Moreover, plastinated tissues avoid the daily exposure of students, academics or technical staff to hazardous substances, because they are odourless and free of toxic chemicals such as formaldehyde, phenol or alcohol (4, 5, 6).

Specimens obtained with the well-known S10 silicone plastination technique (7) are of excellent quality, preserving the original shape and morphology of the specimens along with some flexibility (associated with thinner samples) (8). Plastinated organs have been widely used as a teaching/learning tool in higher education, especially in anatomy. Several previous works have evaluated and even validated the use of plastinated specimens in human and veterinary anatomy practicals (2, 3, 9, 10, 11). Plastination has also been successfully implemented in other disciplines such as zoology (12, 13, 14) and botany (15). A few papers have also referred to the use of plastination to preserve parasites, however they do not provide specific information about their potential use in teaching (12, 16, 17). The validation of plastinated parasites as a teaching tool may help to progressively replace the traditional wet

specimens preserved in toxic fixatives, such as formaldehyde, during practical sessions (17, 18).

The objective of this study was to validate the use of plastinated macroparasites in practical lessons in parasitology, by evaluating the satisfaction and knowledge of students studying for degrees in veterinary medicine and pharmacy.

## Materials and methods

### Specimens

Parasites were obtained from the collection of the Animal Health Department, University of Murcia, Spain: arthropods (*Oestrus ovis*), nematodes (*Parascaris equorum*, *Ascaris suum* and *Macracanthorhynchus hirudinaceus*) and plathelminthes (*Fasciola hepatica*, *Dicrocoelium dendriticum* and *Taenia* sp.) were used (Table I). The specimens were grouped into wet specimens (W, control) fixed in 10% formalin or in ethanol, and plastinated specimens (P) preserved with the standard method of the S10 silicone technique (Biodur® company) (19).

**Table I**

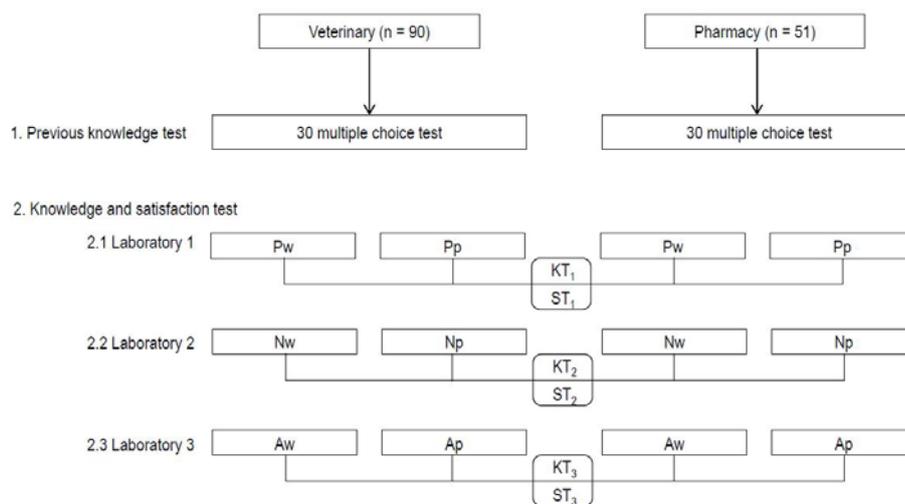
### Plastinated parasites used for practical lessons in veterinary medicine and pharmacy degree programmes

Each parasitic group included specimens of plastinated and non-plastinated specimens, as appropriate

Group	Plastinated specimens
Arthropods	<i>Oestrus ovis</i> larvae (type 2)
	<i>Oestrus ovis</i> larvae (type 3)
	<i>Ascaris suum</i>
Nematodes	<i>Macracanthorhynchus</i>
	<i>hirudinaceus</i>
	<i>Parascaris equorum</i>
Plathelminthes	<i>Dicrocoelium dendriticum</i>
	<i>Fasciola hepatica</i>
	<i>Taenia</i> spp.

## Groups of students

During the 2017/2018 academic year, a total of 141 students of veterinary medicine and pharmacy at the University of Murcia were involved in this project. Information about sex, age and examination failures in previous years was registered to evaluate the influence of these factors on the results obtained. This experiment was performed during three laboratory sessions for each degree subject (Practical 1: plathelminthes, Practical 2: nematodes, and Practical 3: arthropods). The distribution of the study groups and the structure of the assessment of previous knowledge and the knowledge and satisfaction tests were based on previous studies that used plastinated material as a teaching tool (11, 20). The study design of this experiment is represented in Figure 1.



**Fig. 1**

## Study design

The study included a previous knowledge test and the knowledge and satisfaction tests for three laboratories (laboratories 1–3). N: number; Pw: wet plathelminthes; Nw: wet nematodes; Aw: wet arthropods; Pp: plastinated plathelminthes; Np: plastinated nematodes; Ap: plastinated arthropods; KT<sub>1-3</sub>: knowledge test (laboratories 1–3); ST<sub>1-3</sub>: satisfaction test (laboratories 1–3)

In each practical, the number of students involved was 48–51 in pharmacy (34–37 women and 14 men) and 78–90 in the veterinary medicine degree course (65–73 women and 13–17 men). These students were randomly distributed into two groups: P (using plastinated

specimens) and W (using classical wet specimens). For reasons outside of the authors' control, probably influenced by the curiosity of the students about using a new teaching tool (plastinated parasites), the P group had more individuals than the W group.

In order to ensure a blinded study, plastinated or wet specimens were distributed in different sessions of the practical, thus avoiding any possibility of contact between the two experimental groups.

### **Assessment of previous knowledge**

Previous knowledge was tested in order to assess the homogeneity of the groups of students at the beginning of the study. It was an anonymous test with 30 multiple choice questions and four options with only one of them correct. Questions were related to topics including life cycles, geographical distribution, zoonotic conditions and public health relevance, interaction with hosts and economic importance of the parasites.

### **Laboratory sessions**

The three laboratory sessions involved an autonomous evaluation of the structures and morphology of different parasitic groups (plathelminthes, nematodes and arthropods) without time restriction. Upon completion of each session, the students were provided with two individual tests: first, a questionnaire to evaluate knowledge and, second, a satisfaction questionnaire.

### **Knowledge questionnaire**

In the knowledge performance test, each student answered four multiple choice questions about morphological characteristics of specimens of each of the three parasitic groups (Table II), with an equivalent score for each (2.5 points per question, with a maximum rating of 10 points) (Appendix I).

**Table II****Number of students completing the questionnaires**

Laboratory 1 (plathelminthes), Laboratory 2 (nematodes), Laboratory 3 (arthropods), W (wet parasites, control), P (plastinated parasites, experimental group)

Degree	Veterinary		Pharmacy	
Content	W group	P group	W group	P group
Laboratory 1	37	50	20	31
Laboratory 2	37	53	21	27
Laboratory 3	28	50	20	28
Total for study group	102	153	61	86
Total for degree	255		147	
Total questionnaires	402			

**Satisfaction questionnaire**

This questionnaire comprised five items structured as a Likert-type scale with the following topics, related to plastinated or wet parasites:

1. Did the material help you to learn in the practical lesson? (1 [not at all] to 5 [very much]).
2. What is your opinion about the handling of the parasites? (1 [very uncomfortable] to 5 [very comfortable]).
3. Would you like to have more abundant collections and more groups of parasites for use in the practical lessons? (1 [not at all] to 5 [very much]).
4. How do you evaluate the use of parasites as a teaching tool for practical lessons? (1 [dislike it a lot] to 5 [like it a lot]).
5. Do you recommend the use of the same parasites in the practical lessons for the oncoming years? (1 [totally disagree] to 5 [totally agree]).

## Statistical analysis

The statistical software Rstudio v1.0.143 (<http://cran.r-project.org/>) was used to analyse the answers recovered from all questionnaires. Parameters were evaluated for statistical differences using parametric tests (Student's *t* test and calculation of the Pearson correlation coefficient), following prior demonstration that the data had a normal distribution. Significance was set to  $p < 0.05$ .

## Results

### Assessment of previous knowledge

The results of this pre-test showed that the groups of participants in both degree programmes were homogeneous. This meant that both groups started from a similar level of knowledge, so the results obtained in the study were comparable.

### Knowledge questionnaire

Scores were higher in pharmacy (global mean of 6.07 points out of 10) than in veterinary medicine (global mean of 5.75 points out of 10). Among the pharmacy students, the W and P groups obtained an average of 6 and 6.14 points out of 10, respectively. The veterinary students scored 5.7 (W) and 5.8 (P) points out of 10, respectively. Altogether, the average score of the students was 5.9 and 6 out of 10 for the W and P groups, respectively. However, differences between scores obtained in W and P groups were not statistically significant in any of the above-mentioned comparisons.

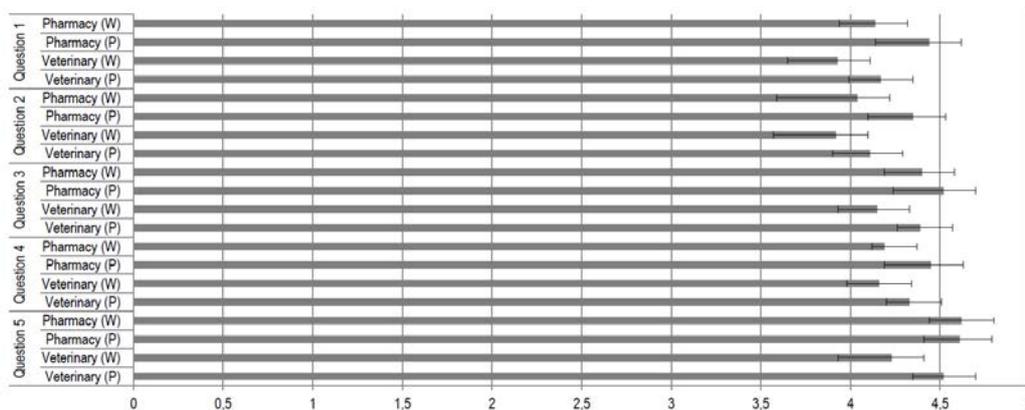
The results were also separately evaluated for each practical lesson (plathelminthes, nematodes and arthropods). No differences for any phylum were observed between W and P groups among either pharmacy or veterinary students ( $p > 0.05$ ).

The analysis of the students' characteristics (sex, age and repeater condition) revealed a mild positive correlation ( $r = 0.082$ ) between veterinary students repeating the subject and the knowledge scores, although there were no statistically significant differences when

considering the use of plastinated or non-plastinated parasites in practical lessons ( $p = 0.109$ ).

### Satisfaction questionnaire

The majority of students using plastinated specimens (96.7%) selected high scores for all the items (average > 4 points out of 5) in the satisfaction questionnaire, while only 73.3% of the students using conventional wet material marked that option ( $p > 0.05$ ) (Fig. 2).



**Fig. 2**

**Mean values ( $\pm$  standard deviation) provided by students in relation to W (wet parasites) and P (plastinated parasites) groups for each satisfaction question**

The answers of pharmacy students showed positive statistically significant differences related to the handling advantages (question 1;  $p < 0.05$ ) and the relevance for learning (question 2;  $p < 0.05$ ) of plastinated parasites. Moreover, veterinary students valued positively the use of plastinated parasites as a teaching tool during practical lessons (question 4;  $p < 0.05$ ) (Fig. 2).

A more robust statistical analysis, with an increase in sample size obtained by combining all parasitic groups, showed that plastinated material was highest rated (question 4;  $p < 0.05$ ) by pharmacy students.

In general, the pharmacy and veterinary students expressed a preference for using plastinated arthropods. All experimental groups of parasitic specimens obtained scores above their homologues in the control group. However, neither plastinated nor conventional wet material showed statistically significant differences with regard to the parasitic groups, the degree programme or the student's age, sex or repeater condition.

A low positive correlation ( $r = 0.061$ ) was observed between the knowledge test and the general satisfaction level for plastinated and non-plastinated parasites among students of both pharmacy and veterinary medicine degrees ( $p = 0.098$ ).

## Discussion

The results obtained in this study validate the use of plastinated parasites as an alternative teaching/learning tool during parasitology practical lessons in veterinary medicine and pharmacy degrees. Similarly, in other scientific areas of higher education (e.g. anatomy) publications have demonstrated that plastinated specimens are a valid alternative to material fixed with formaldehyde (11, 20). When aiming to evaluate a new teaching tool, it is necessary to ensure the homogeneity of groups (previous knowledge assessment) as well as to compare the experimental data with control information in order to obtain objective results in the analysis and interpretation of the evaluation tests (knowledge and satisfaction questionnaires). Students using plastinated material obtained a better average score in the knowledge test than those using conventional wet specimens, although there were no statistically significant differences. This result may be related to the easy handling of plastinated parasites which, according to previous studies, could be a key factor in facilitating the study of the morphological characteristics of parasites (17, 21).

The use of plastinated parasites gained higher acceptance than the traditional wet material in the satisfaction questionnaire, although both pharmacy and veterinary students marked an average score of more than 4 points out of 5 for all the items. Satisfaction with plastinated arthropods obtained the highest score, which could be related to the

problems of fragility and management in these species when compared with plathelminthes and nematodes (22).

According to our results, parasite plastination is a promising alternative for the future, and it appears to be an interesting option for preserving teaching tools for routine use in practical sessions in parasitology.

This work is a preliminary study for future experiments in which the number of plastinated parasites species should be greater, in order to work with more specimens whose identification is important for diagnosis, treatment and prophylaxis.

The traditional method of fixing specimens with formaldehyde is considered a significant health hazard. On the other hand, the cost of plastination equipment and chemicals is much greater. Moreover, it is necessary to have good knowledge of the plastination technique to obtain high quality plastinated specimens. The advantages of incorporating plastinated parasites in the teaching material of parasitology include provision of a dry, odourless, non-irritating, non-carcinogenic and non-toxic material, ensuring safe procedures in higher education (6, 17, 20, 21, 23, 24). Moreover, the easy storage and conservation of plastinated material is an additional incentive because it ensures a durable teaching tool for use in educational centres (11, 20, 21).

## Conclusions

The use of plastinated parasites for teaching purposes allows students to learn morphological structures in parasitology, with no demonstrated differences from wet formaldehyde-preserved specimens. Moreover, this methodology achieved high satisfaction scores with regard to the students' perception of the quality of the specimens when compared with those preserved in formaldehyde.

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## Résumé français: titre

### Résumé

### Mots-clés

## Resumen español: título

### Resumen

### Palabras clave

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## Appendix I

### Assessment of knowledge: multiple choice questions about plathelminthes, nematodes and arthropods provided to the students

#### Plathelminthes

1. Which structure/s could be identified in the anterior region of *Fasciola hepatica* and *Dicrocoelium dendriticum*?
    - a) Four prominent lips
    - b) Two keratinised hooks
    - c) An oral sucker
    - d) A crown of hooks
  
  2. Which structure/s could be found in the ventral region of *Fasciola hepatica* and *Dicrocoelium dendriticum*?
    - a) A ventral nerve cord
    - b) A ventral sucker
    - c) Genital–anal setae
    - d) Keratinised hooks
  
  3. Which of the following definitions fits *Fasciola hepatica*?
    - a) Segmented trematode with foliaceous shape and length of approximately 3 cm
    - b) Non-segmented trematode, foliaceous aspect and length of approximately 0.5 cm
    - c) Non-segmented trematode, foliaceous aspect and length of approximately 3 cm
    - d) Segmented trematode, oval aspect and length of approximately 3 cm
-

4. The shape of the proglottids of *Taenia* spp. could be defined as:
  - a) An oval shape
  - b) The posterior edge is always wider than the anterior one
  - c) The posterior edge is always narrower than the anterior one
  - d) A rectangular shape

### Nematodes

1. Which structure/s could be easily identified in the anterior region of *Parascaris equorum*?
  - a) Three prominent lips
  - b) Two large buccal hooks
  - c) An oral sucker
  - d) A double crown of hooks
2. The cuticle of *Ascaris suum*:
  - a) Is covered by flakes
  - b) Has a smooth surface with small transverse striations
  - c) Has spicules directed backwards along its entire length
  - d) Has deep segmentations that divide the body into four parts
3. Which structure can be identified in the anterior region of *Macracanthorhynchus hirudinaceus*?
  - a) A proboscis with hooks
  - b) Two long fimbriae (2 cm approx.)
  - c) A sucker with four keratinised hooks

- d) Three lobed lips
- 4. The cuticle of *Macracanthorhynchus hirudinaceus*:
  - a) Is entirely smooth
  - b) Has transverse striations along its entire length
  - c) Has spines directed backwards along its entire length
  - d) Is covered with scales

#### Arthropods

- 1. What is the main difference between the second (L2) and third (L3) stage larvae of *Oestrus ovis*?
  - a) They are very similar, and a morphological analysis of their spiracles is necessary to distinguish them
  - b) L2 are smaller and whiter than L3, which are larger and have dark transverse striations along their length
  - c) L2 are larger and with dark transverse striations, while L3 are smaller and white
  - d) L2 have two suckers in the anterior region that differentiate them from the L3
- 2. The anterior region of *Oestrus ovis* larvae (L2 and L3) has:
  - a) Two keratinised hooks
  - b) A single crown of hooks
  - c) A large sucker
  - d) Three prominent lips

3. The surface of *Oestrus ovis* larvae (L3):
  - a) Has squamous/verrucous structures over the entire length
  - b) Is smooth, without remarkable structures
  - c) Has macroscopic sensorial fimbriae along its length
  - d) Has rows of spines and dark transverse bands in some segments
  
4. The posterior region of *Oestrus ovis* larvae (L2 and L3) has:
  - a) A powerful sucker to increase their adherence to the host
  - b) Two dark and circular structures called spiracles
  - c) Four thick terminal spines
  - d) Two long spicules (2 cm)