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Phenotypic analysis of adults and eggs of *Fasciola hepatica* by computer image analysis system

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Abstract

Knowledge of the morphological phenotypes of the liver fluke *Fasciola hepatica* (Trematoda: Digenea) is analysed. The influence of parasite age on its dimensions, the adult fluke growth model, variation in a biometric variable versus time, and variation in a biometric variable versus another biometric variable (allometric model) are revised. The most useful allometric model appears to be $(y_{2m} - y_2)/y_2 = c\,[(y_{1m} - y_1)/y_1]^b$, where y_1 = body area or body length, y_2 = one of the measurements analysed, y_{1m}, y_{2m} = maximum values towards which y_1 and y_2, respectively, tend, and c, b = constants. A method based on material standardization, the measurement proposal and allometric analysis is detailed. A computer image analysis system (CIAS), which includes a colour video-camera connected to a stereomicroscope (for adult studies) and a microscope (for egg studies), facilitates the processing of digital imaging. Examples of its application for the analysis of the influence of different factors on the liver fluke phenotype are shown using material from the Northern Bolivian Altiplano, where human and domestic animal fascioliasis is caused by *F. hepatica* only. Comparisons between the development of livestock fluke populations from highlands and lowlands are discussed and the relationships between host species and liver fluke morphometric patterns is analysed.

Introduction

Fascioliasis is a highly pathogenic disease caused by two trematode species that belong to the genus *Fasciola*: *F. hepatica* Linnaeus and *F. gigantica* Cobbold (Mas-Coma & Bargues, 1997). Fascioliasis has been shown to be an important public health problem (Mas-Coma *et al.*, 1999a, 2000), with human cases reported in countries on five continents (Esteban *et al.*, 1998) and human endemic areas ranging from hypo- to hyperendemic (Mas-Coma *et al.*, 1999b). The estimated worldwide human infection is between 2.4 (Rim *et al.*, 1994) and 17 million (Hopkins, 1992), while the population at risk is estimated to be 180 million (WHO, 1995). Due to the large colonization capacities of its causal agents and vector species, fascioliasis is a disease that has a very high potential for expansion. It is emerging or re-emerging in many countries and its prevalence, intensity and geographical distribution are increasing (Mas-Coma, 2004). Today, fascioliasis is the vector-borne disease presenting the widest latitudinal, longitudinal and altitudinal distribution known (Mas-Coma *et al.*, 2003).

Certain biological differences between *F. hepatica* and *F. gigantica* influence the prevalence of these trematodes in different regions. *Fasciola hepatica* is more prevalent in temperate zones and, therefore, is dominant in Europe, the Americas and Oceania (Over, 1982), while *F. gigantica* is environmentally adapted to the tropical and humid zones that are predominant in Africa and Asia (Chen & Mott, 1990). The distribution of *F. hepatica* and *F. gigantica* sometimes overlaps in parts of Asia and Africa and this makes it difficult to identify the particular species involved, which is often referred to simply as *Fasciola* sp.

The morphological phenotype of a living organism is an extremely complex and dynamic system. Morphology has been the most frequently used criterion for systematic studies on fasciolids. Different species and/or subspecies described have been differentiated by analysing adult worms and eggs from domestic animal hosts. Many have

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been invalidated (Kendall, 1965). The classification of *Fasciola* species is particularly based on differences in the shape and size of adults and eggs, whose values overlap in some cases. From a phenotypic point of view, one difference between adults of *Fasciola* and those of other liver-fluke species is that most of the structures (ovary, testis and intestinal caeca) are branched (Jackson, 1921; Watanabe, 1962; Bergeon & Laurent, 1970). For this reason, 'classical' measurements of the body alone such as body length or body width appear to be insufficient to characterize the phenotype of these species. Only a few studies have quantitatively assessed the influence of different factors on adult and egg morphology of these two fasciolids (Boray, 1969; Akahane, *et al.*, 1970, 1974; Srimuzipo *et al.*, 2000).

The present paper aims to analyse data on phenotypes of the liver fluke, *F. hepatica* using computer image analysis based on standardization of the study material, a measurement proposal and an allometric analysis. Examples of its application for the analysis of the effect of different factors on the liver fluke phenotype is detailed using materials mainly from the Northern Bolivian Altiplano, where human and domestic animal fascioliasis is caused by *F. hepatica* (Valero *et al.*, 1999; Mas-Coma *et al.*, 2001) only. The prevalences and intensities of human fascioliasis in this geographical area are the highest known (Esteban *et al.*, 1999; Mas-Coma *et al.*, 1999c), and *Galba truncatula* (Gastropoda: Lymnaeidae) is the only intermediate host species (Bargues & Mas-Coma, 1997; Mas-Coma *et al.*, 2001). The materials used were compared with materials originating from Europe, especially from the Mediterranean island of Corsica, France and Valencia, Spain.

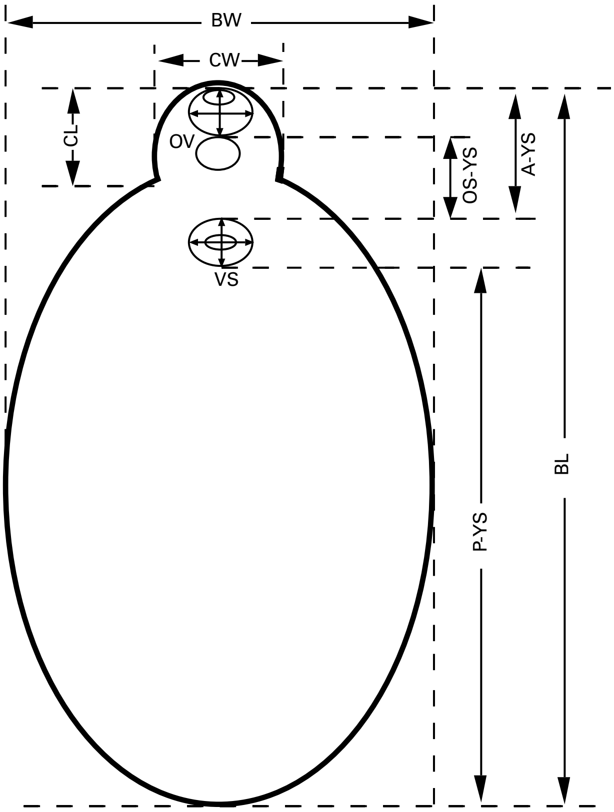

Fig. 1. Standardized measurements of a *Fasciola hepatica* adult.

Standardization of study material

Material must be fixed using the same technique. Adult worms analysed in this paper were fixed in Bouin's solution between slide and coverglass with little pressure, stained with Grenacher's borax carmine and mounted in Canada balsam. Eggs were analysed without fixation in filtered faeces or bile.

Standardization of methodology

Image analysis and measurement standardization

A computer image analysis system (CIAS), with a colour video camera connected to a stereomicroscope (for adult worms) and microscope (for eggs), facilitates the processing of digital imaging. Uni-, bi- and tridimensional measurements can be obtained in selected digital images, furnishing ratios of interest for concrete biometric parameters.

With reference to fasciolids, the standardized methodology for measurements of adults of *F. hepatica* was proposed by Valero *et al.* (1996, 2001a), based on the method for brachylaimid trematodes proposed by Mas-Coma *et al.* (1984). Adult measurements for *F. hepatica* adults are (fig. 1): (i) linear biometric characters, namely, body length (BL), body width (BW), perimeter (P), cone length (CL), cone width (CW), oral sucker maximum (OS Ø max) and opposite (= following the axis perpendicular to the maximum diameter) (OS Ø op) diameters, ventral sucker maximum (VS Ø max) and opposite (VS Ø op) diameters, distance between anterior end of body and ventral sucker (A-VS), distance between suckers (OS-VS), and distance between ventral sucker and posterior end of body (P-VS); (ii) areas, namely, body area (BA), oral sucker area (OSA), ventral sucker area (VSA) and uterus area (UA); and (iii) ratios, namely, sucker ratio (OS/VS), BL/BW ratio and BL/P-VS ratio. Egg measurements proposed for fasciolids are: (i) linear measurements, namely, egg length (EL), egg width (EW) and egg perimeter (EPe); (ii) areas, i.e. egg area (EA); and (iii) ratios, i.e. EL/EW ratio.

Growth law models

Development has long been known to be a stable process. Waddington (1957) coined the term canalization for this feature. Canalization suggests that development follows restrictive ways, or creodes (Waddington, 1957; Alberch *et al.*, 1979; De Renzi, 1997; Rice, 1998). The concept of ontogenetic trajectory can provide a quantitative representation of the creodes, as suggested by Alberch *et al.* (1979). These authors considered ontogenetic trajectory to be the change in size and shape of a morphological structure with age. There is a small number of growth law models which fit a large spectrum of magnitudes defined in morphological structures. Three of these models are common: the exponential model, the saturated model (also known as the Bertalanffy model) and the logistic model (Alberch *et al.*, 1979; Valero, *et al.*, 1991; De Renzi, 1997; Rice, 1998). Valero *et al.* (1996, 1998) have shown in a rodent experimental model that traditional morphometric measurements used for adults of *F. hepatica* follow a logistic growth model with respect to time:

$$y = y_m/[1 + z_0\, exp(-kt)] \quad (1)$$

where y_m is the maximum value attained by a biometric variable y; z_0 and k are constants or parameters of the trajectories; and z_0 is related to the initial value of the magnitude y, i.e. the initial conditions y_0 at $t = 0$, and it exhibits a different expression in each case. This logistic equation makes it possible to analyse the influence of adult liver fluke age on its dimensions, i.e. the variation of a biometric variable versus time. Its application is only possible on experimental populations.

This model describes the variation in adult dimensions with time, from parasite migration to the adult location in the bile duct. The logistic model which represents body growth and development is characterized by two phases (Valero *et al.*, 1998): the 'exponential' part of logistic growth corresponds to body development during migration in the abdominal cavity and liver parenchyma, as well as to development and sexual maturation in the biliary duct system up to the onset of egg production. From this moment, development follows the 'saturated' part of logistic growth. In detail, the pattern of fluke development comprises two periods: (i) gonadal differentiation, appearance of uterine eggs and the onset of ovoposition occur in the 'exponential' period; and (ii) considerable persistence of growth after sexual maturity, followed by gradually stationary growth thereafter, in the 'saturated' period. The ovoposition is the inflection point of the logistic growth marking the end of the 'exponential' period and the beginning of the 'saturated' period. This model implies that the morphometric development of an *F. hepatica* adult is not unlimited but 'damped' and does not exceed certain characteristic maxima (y_m).

Allometric models

For accurate morphometric comparisons, increases in different biometric parameters which occur during digenean development within the definitive host according to growth laws must be taken into account (Dawes & Hughes, 1964; Valero *et al.*, 1996, 1998). If adult populations of different ages are studied, morphometric differences attributable to age can appear. When studying natural populations, only the allometric growth of a given biometric measurement as a function of another biometric measurement can be calculated (Valero *et al.*, 1991, 1996, 1999).

An alternative allometric function for *F. hepatica* adults (based on logistic growth law versus time) for studying the relationship between two morphometric variables y_1 and y_2 in fasciolids has been employed (Valero *et al.*, 1996, 1999):

$$(y_{2m} - y_2)/y_2 = c[(y_{1m} - y_1)/y_1]^b \quad (2)$$

where: y_1 = BA or BL; y_{1m} is the maximum value towards which y_1 tends; y_2 = BL, BW, Pe and P-VS; y_{2m} is the maximum value towards which y_2 tends; and c and b are constants.

BL and BA may be selected as age measurements for the natural population, taking into account the general adult morphology of *F. hepatica*. To calculate asymptotic values of y_m, a procedure consisting of simultaneously testing successive values for y_{1m} and y_{2m} with the least squares residual (sse) may be used. This function was successfully applied to experimental material obtained in rats, in adult material between 30 and 261 days post-infection, i.e. the model is an adequate representation of the development of adult liver flukes (Valero *et al.*, 2001b).

Statistical analyses

If log e transformations are applied, differences in allometric curves are sought by analysis of covariance (ANCOVA) (one-way analysis of variance design with one covariate) using initial transformations of BA or tBL as a covariate. Adjusted non-linear curves are tested using the correlation coefficient r^2 and sse. Comparison of average egg measurements from different host species can be carried out using the one way ANOVA and three post hoc tests. Values are considered statistically significant when $P < 0.05$. Data processing is carried out with SPSS.

Development comparison in adult *Fasciola hepatica* between livestock liver fluke populations from highlands and lowlands

It has been known for many years that animals born and living at high altitudes present morphological and physiological characteristics different from those at low altitudes. In the high altitude environment, oxygen and air density decrease, temperature and humidity are low and there is an increase in radioactivity. There are several studies which show that vertebrates inhabiting high altitude zones exhibit changes such as hypoxia (Levine *et al.*, 1988), alterations in immune responses (Sarybaeva *et al.*, 1988), elevated haematocrit levels, differences in blood oxygen pressure values and blood viscosity (Sakai *et al.*, 1984) and elimination of dissolved gases, especially N_2, from the blood (Hirai *et al.*, 1988).

With reference to parasites, studies on phenotype variability in helminth populations parasitizing hosts living under the extreme environmental conditions of very high altitude are unfortunately scarce. With respect to *F. hepatica*, Valero *et al.* (1999, 2001a) analysed the influence of altitude on the development of the liver fluke, by comparing populations obtained from the same livestock species naturally-infected but born and living in the highlands (Bolivian Altiplano) and lowlands (European Mediterranean area).

Body development

Valero *et al.* (1999) made a morphometric comparison between natural liver fluke populations from highland sheep versus lowland sheep. Morphometric values of both Bolivian and Spanish materials are shown in table 1. Samples analysed include the largest worm variability (different stages of maturity, body size and uteri with and

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Table 1. Comparative morphometrical data of *Fasciola hepatica* adults and eggs from naturally infected sheep, cattle and pigs from Spain and the Northern Bolivian Altiplano and Wistar rats experimentally infected with Altiplanic definitive host species isolates. All values are shown as range and mean ± standard error.

	Sheep Spain	Sheep Bolivia	Cattle Bolivia	Pig Bolivia	Exp. rat Bolivia
Body area, BA (mm^2)	59.40–197.24 126.153 ± 3.30	6.08–216.77 84.70 ± 46.96	19.06–196.35 114.31 ± 34.25	42.47–182.03 101.69 ± 32.65	6.90–191.79 89.76 ± 50.03
Body length, BL (mm)	12.13–26.51 19.68 ± 0.37	4.90–31.11 16.10 ± 4.80	8.92–28.74 19.07 ± 3.45	10.03–24.87 16.91 ± 3.00	4.26–23.37 14.24 ± 4.59
Body width, BW (mm)	6.87–10.82 9.02 ± 0.11	1.58–12.55 7.11 ± 2.27	3.16–12.95 8.39 ± 1.51	5.21–11.45 8.50 ± 1.55	1.90–12.95 8.11 ± 3.08
BL/BW ratio	1.13–2.95 4.86 ± 0.56	1.33–4.17 2.33 ± 0.44	1.40–3.48 2.30 ± 0.37	1.50–2.68 2.02 ± 0.27	1.18–2.99 1.85 ± 0.36
Perimeter, P (mm)	31.240–61.320 46.980 ± 0.757	12.39–68.35 43.13 ± 12.54	23.60–67.73 52.03 ± 8.33	30.23–66.39 47.32 ± 7.79	12.28–62.91 40.33 ± 13.12
Cone length, CL (mm)	1.31–2.75 1.94 ± 0.05	0.80–3.04 2.06 ± 0.31	1.38–3.06 2.11 ± 0.31	1.60–3.38 2.33 ± 0.33	0.85–3.08 1.95 ± 0.42
Cone width, CW (mm)	2.29 ± 3.71 2.99 ± 0.05	1.20–3.90 2.46 ± 0.43	1.48–3.75 2.76 ± 0.41	2.03–4.47 3.18 ± 0.47	1.03–3.73 2.60 ± 0.72
Oral sucker area, OSA (mm^2)	0.21–1.03 0.46 ± 0.02	0.08–0.66 0.34 ± 0.09	0.18–0.57 0.37 ± 0.07	0.22–0.68 0.43 ± 0.09	0.07–0.39 0.25 ± 0.09
Ventral sucker area, VSA (mm^2)	0.66 ± 1.89 1.08 ± 0.04	0.15–1.23 0.71 ± 0.19	0.37–1.70 0.80 ± 0.15	0.45–1.25 0.88 ± 0.17	0.12–1.06 0.63 ± 0.26
OS/VS ratio	0.04–0.87 0.44 ± 0.01	0.25–0.70 0.49 ± 0.08	0.22–0.74 0.47 ± 0.08	0.30–0.70 0.49 ± 0.08	0.19–0.71 0.42 ± 0.09
Distance between anterior end of body and ventral sucker, A-VS (mm)	1.13–2.89 2.02 ± 0.06	0.79–3.35 2.11 ± 0.36	1.49–2.98 2.27 ± 0.28	1.62–3.35 2.36 ± 0.35	0.90–2.80 1.97 ± 0.45
Distance between ventral sucker and posterior end of body, P-VS (mm)	8.23–22.35 6.39 ± 0.35	3.16–27.39 13.03 ± 4.45	6.63–24.95 15.81 ± 3.19	7.58–20.18 13.52 ± 2.64	2.76–19.58 11.41 ± 4.03
*Uterus area, UA (mm^2)	0.87–13.65 5.90 ± 2.224	0.22–7.15 3.08 ± 1.44	1.22–8.69 4.26 ± 1.88	0.890–8.82 3.87 ± 1.88	–
Egg length, (EL) (μm)	–	114.77–151.16 130.80 ± 7.14	105.29–155.88 132.01 ± 10.46	73.84–148.60 123.78 ± 11.30	98.09–144.18 124.57 ± 7.79
Egg width, (EW) (μm)	–	65.51–81.42 72.59 ± 3.89	61.67–82.47 71.14 ± 4.40	58.06–82.55 71.83 ± 4.36	56.94–80.8 67.64 ± 3.40
Egg perimeter, (EPe)(μm)	–	294.22–368.19 327.63 ± 15.02	270.64–422.86 339.98 ± 33.43	247.77–360.05 313.68 ± 20.92	256.79–352.14 308.81 ± 15.11
Egg area, (EA)(μm^3)	–	5998.17–8608.54 7238.04 ± 532.77	5286.50–9676.81 7170.17 ± 802.50	3988.68–8626.87 6836.98 ± 820.36	4836.23–7982.26 6380.05 ± 510.77
EL/EW ratio		1.48–2.15 1.81 ± 0.14	1.56–2.28 1.86 ± 0.16	1.06–2.05 1.73 ± 0.16	1.38–2.21 1.85 ± 0.15

*Only in gravid specimens

without eggs). Function allometry shape shows only slight significant differences between Bolivian and Spanish populations.

Despite the geographical distance and the marked environmental differences between the Bolivian Altiplano and the Spanish regions concerned, in adult flukes only a smaller size in the majority of the parameters in the Bolivian material was found. Differences in the shape of allometries between both populations studied are likely to be due to geographical variability related to altitude effects or genetic isolation. The existence of only slight significant morphometric differences is surprising when taking into account the great geographic distance between both liver fluke populations and the markedly different conditions imposed by very high altitudes.

Uterus development

To analyse the influence of altitude on the development of the uterus of *F. hepatica*, natural liver fluke populations from highland sheep versus lowland sheep

Table 2. Allometric function[a] obtained from a logistic model with respect to time in adult worms of *Fasciola hepatica* from naturally infected sheep, cattle and pigs of the Northern Bolivian Altiplano (highland) and European Mediterranean area (lowland) and Wistar rats experimentally infected with Altiplanic definitive host species isolates.

	y_{1m}	y_{2m}	c ± SE	b ± SE	r^2	sse
Population from sheep (lowland)						
BA/BL	201.00	27.50	0.83300 ± 0.06500	0.60800 ± 0.02200	0.82	64.57900
BA/BW	201.00	10.90	0.75900 ± 0.10300	0.30000 ± 0.01700	0.56	14.61700
BA/P	201.00	64.00	0.79500 ± 0.04200	0.54300 ± 0.01300	0.91	137.66400
BA/P-VS	201.00	22.90	0.98600 ± 0.07700	0.65000 ± 0.02700	0.83	55.54600
BA/UA	256.00	14.00	0.67600 ± 0.11300	1.61200 ± 0.11500	0.41	84.20000
BL/BW	27.50	10.90	0.31300 ± 0.04100	0.44900 ± 0.13600	0.31	26.41400
BL/P	27.50	64.00	0.87300 ± 0.02900	0.81400 ± 0.02100	0.96	57.13300
BL/P-VS	27.50	22.90	1.16300 ± 0.03100	1.14800 ± 0.03100	0.97	7.61800
Population from sheep (highland)						
BA/BL	226.00	32.21	0.69633 ± 0.00936	0.59565 ± 0.01231	0.92	525.09938
BA/BW	226.00	12.56	0.45095 ± 0.00949	0.77504 ± 0.01861	0.91	137.29843
BA/P	226.00	75.00	0.45531 ± 0.00663	0.72091 ± 0.01265	0.95	2422.36546
BA/P-VS	226.00	27.40	0.74551 ± 0.01181	0.65005 ± 0.01508	0.91	528.72614
BA/UA	327.00	7.60	1.13000 ± 0.09700	0.52900 ± 0.05200	0.60	94.31000
BL/BW	32.21	12.56	0.71615 ± 0.01908	1.14647 ± 0.05173	0.71	417.26706
BL/P	32.21	75.00	0.69948 ± 0.00730	1.19704 ± 0.02050	0.95	2222.73147
BL/P-VS	32.21	27.40	1.10674 ± 0.00304	1.09367 ± 0.00522	1.00	23.10781
Population from cattle (highland)						
BA/BL	217.99	28.90	0.53601 ± 0.00939	0.72433 ± 0.02782	0.83	330.73986
BA/BW	217.99	12.96	0.56795 ± 0.01135	0.65308 ± 0.03129	0.76	91.24020
BA/P	217.99	70.40	0.35731 ± 0.00579	0.87204 ± 0.02497	0.90	1149.03709
BA/P-VS	217.99	24.96	0.60566 ± 0.01152	0.75077 ± 0.03075	0.82	309.00564
BA/UA	245.00	8.70	1.03300 ± 0.18500	1.01400 ± 0.09100	0.45	112.03000
BL/BW	28.90	12.96	0.79676 ± 0.03926	0.55887 ± 0.06122	0.35	248.62788
BL/P	28.90	70.40	0.72683 ± 0.01319	1.15973 ± 0.02816	0.93	838.30993
BL/P-VS	28.90	24.96	1.15770 ± 0.00867	1.03782 ± 0.01013	0.99	19.56255
Population from pig (highland)						
BA/BL	196.99	28.10	0.66963 ± 0.01136	0.60411 ± 0.02582	0.87	124.74074
BA/BW	196.99	11.56	0.35695 ± 0.01081	0.92887 ± 0.04613	0.84	37.61648
BA/P	196.99	66.81	0.39914 ± 0.00630	0.82732 ± 0.02417	0.94	396.95759
BA/P-VS	196.99	23.80	0.77306 ± 0.01514	0.60484 ± 0.02977	0.83	125.07542
BA/UA	525.00	8.90	1.72000 ± 0.28700	0.12500 ± 0.04800	0.43	136.05000
BL/BW	28.10	11.56	0.57125 ± 0.03156	1.14066 ± 0.11729	0.53	111.84936
BL/P	28.10	66.81	0.66877 ± 0.00950	1.28967 ± 0.03065	0.96	259.26182
BL/P-VS	28.10	23.80	1.16492 ± 0.00675	1.01976 ± 0.01021	0.99	5.92489
Population from rat (highland isolates)						
BA/BL	194.20	23.40	0.51160 ± 0.01138	0.63296 ± 0.01764	0.96	85.42255
BA/BW	194.20	13.40	0.48832 ± 0.01311	0.79136 ± 0.02331	0.96	36.01654
BA/P	194.20	64.40	0.45986 ± 0.01008	0.67723 ± 0.01706	0.97	534.69534
BA/P-VS	194.20	19.60	0.57499 ± 0.01456	0.65897 ± 0.02134	0.95	83.05153
BL/BW	23.40	13.40	1.04654 ± 0.05322	1.15717 ± 0.06535	0.85	132.36810
BL/P	23.40	64.40	0.91959 ± 0.02288	1.05867 ± 0.03182	0.95	772.93706
BL/P-VS	23.40	19.60	1.15781 ± 0.00882	1.04177 ± 0.00926	1.00	6.51515

b, c = constants; SE = standard error; r^2 = adjusted; y_m = maximum value of biometric characters in the allometric model; sse = least squares residual in both models.

[a] $(y_{2m} - y_2)/y_2 = c[y_{1m} - y_1]/y_1]^b$

BA, body area; BL, body length; BW, body width; P, perimeter; P-VS, distance between ventral sucker and posterior end of body; UA, uterus area.

and highland cattle versus lowland cattle were compared separately by Valero *et al.* (2001a). The samples analysed include only gravid specimens but with different gravid stages which represent the process of uterus development.

Morphometric values of BA and UA in Bolivian and Spanish adult worms are shown in table 1. Function allometry shape (BA/UA) was significantly different between Bolivian and European populations (e.g. adults worms obtained from sheep, see table 2). These results in both sheep and cattle demonstrate that the Bolivian highland liver fluke populations proved to have a smaller uterus size than that of the European lowland liver fluke populations. Although it cannot be disregarded that to some extent this may be attributable to intraspecific variability not related to altitude influence, a divergence between highland and lowland populations in uterus size is difficult to understand, as the evolutionary advantages are not easily envisaged. However, if the uterus in *Fasciola* is considered mainly a storage organ (in fasciolids, eggs are laid unembryonated, the miracidium beginning its development inside eggs once in freshwater), the shortening of the uterus length would allow faster egg-laying moving towards a continual egg-laying process. In the Northern Bolivian Altiplano, climatic conditions, freshwater body characteristics and lymnaeid ecology enable fascioliasis transmission to take place throughout the year (Fuentes *et al.*, 1999; Mas-Coma *et al.*, 1999c), so that egg storage is *a priori* not needed as in the northern hemisphere latitudes where fascioliasis transmission is typically seasonal. Further research is presently being carried out to ascertain whether these morphometric differences detected in the uterus of Bolivian *F. hepatica* adults are associated with biological differences.

Relationships between host species and liver fluke morphometric patterns

The influence of host species on morphometric patterns of *F. hepatica* adults and eggs has already been demonstrated in Northern Bolivian Altiplano liver fluke populations (Valero *et al.*, 2001a,b), which is explained below. In this geographical area, sheep, cattle, pigs and donkeys may be considered reservoir host species (Hillyer *et al.*, 1996; Buchon *et al.*, 1997; Mas-Coma *et al.*, 1997, 1999c; Grock *et al.*, 1998).

Liver fluke adults and eggs from naturally infected livestock

Valero *et al.* (2001b) quantitatively characterized the influence of host species on morphometric traits of *F. hepatica* adults (from sheep, cattle and pigs) and eggs (from sheep, cattle, pigs and donkeys) in natural liver fluke populations. Samp]les analysed include the largest worm variability (different stages of maturity, body size and uteri with and without eggs).

Morphometric values of Bolivian *F. hepatica* adults from sheep, cattle and pigs are shown in table 1. Table 2 gives the parameters of the allometric function [2] obtained for seven pairs of variables studied for each host species.

The literature offers contradictory data on the larger or smaller size of liver flukes from sheep and cattle. There have been occasional comments suggesting that flukes from cattle are larger than those from sheep, but no conclusive data have been presented (Panaccio & Trudgett, 1999). Dixon (1964) states that flukes in sheep grow faster, more uniformly and reach a larger size than in cattle. The allometric study of Valero *et al.* (2001b) shows the presence of certain morphological traits that characterize the *F. hepatica* adult in different host species. Although morphometric values of adult liver flukes from sheep, cattle and pigs overlap in natural populations (table 1), results obtained show allometric differences. Maximum values obtained from measurements vary in liver flukes from the three host species (table 2). In adult worms from sheep, the maximum BL and P-VS are higher than in adult worms from cattle and pigs. In adults from cattle, maximum BW is higher than in adults from sheep and pigs. In adults from pigs, values of y_m are smaller than those in adults from sheep and cattle. These results coincide with those of Akahane *et al.* (1974) on *Fasciola* sp. adults in Japan. These authors reported that BL, BW and weight were lower in liver flukes from pigs than in fasciolids from other hosts such as sheep or cattle.

Valero *et al.* (2001b) analysed the morphometric values of *F. hepatica* eggs from naturally-infected Altiplanic Bolivian sheep, cattle, pigs and donkeys (table 1). Significant differences (ANOVA) ($P < 0.05$) were detected in EL, EW, EPe, EA and EL/EW between eggs from sheep, cattle, pigs and donkeys, as well as rats. Significant differences were obtained by comparing each egg measurement in pairs of definitive host species. The EL/EW ratio showed differences in all domestic animal pairs. Valero *et al.* (2002) demonstrated that eggs shed by both naturally and experimentally infected rodents (wild *Mus musculus* and *Rattus rattus*, and *R. norvegicus* Wistar laboratory strain) are smaller in size than those shed by naturally infected cattle from the same European region.

Liver fluke adults and eggs from experimentally infected rats

Valero *et al.* (2001b) experimentally verified whether or not adult and egg morphometric characteristics associated with a natural definitive host species remain unchanged after passing through a rodent definitive host model. For this purpose, adults and eggs were experimentally obtained in Wistar rats infected with fluke isolates from Altiplanic sheep, cattle and pigs.

In adults, no significant differences in the seven pairs of variables were detected between the different isolates analysed. Thus, the allometry of rat worms appears to be independent of the isolate. Significant differences were obtained when comparing each function allometry in pairs of definitive host species (sheep, cattle or pig versus rat).

Adults of *F. hepatica* obtained in Wistar rats exhibit a proportional relationship between trematode body size and host mass. As pointed out by Poulin (1997), host mass probably correlates with the space available for parasites in various organs, which may place physical constraints upon trematode body size. In the rat, the common bile duct is the usual habitat for the liver fluke in the latent phase, and although the common bile duct in the rat undergoes a patent hyperplasia (Foster, 1981), it severely limits the parasite size. Smaller body measurements in flukes obtained in rats were evident in all maximum values analysed except BW. Thus, the artificial mechanical cap, introduced by using rats as hosts in liver fluke development studies, must not be forgotten when making comparisons between liver fluke livestock isolates obtained in rats.

Morphometric values of *F. hepatica* eggs shed by Wistar rats infected with sheep, cattle and pig isolates are shown in table 1. No significant differences were detected in EL, EW, EPe, EA or EL/EW when comparing the three isolates. Thus, egg morphometry appears to be independent of the isolate.

Variations have been observed in the size of *F. hepatica* eggs from different hosts and geographical locations (Tinar, 1984). Valero *et al.* (2001b) revealed that the final host species decisively influences the size of *F. hepatica* adults and eggs even within the same endemic area. Moreover, these experimental studies prove that this influence does not persist in a rodent definitive host model. These findings indicate that in endemic areas in which *F. hepatica* and *F. gigantica* coexist thorough studies on egg morphometry must be carried out.

Uterus development

To analyse the influence of host species on the development of the liver fluke uterus in the highlands, Bolivian Altiplanic natural liver fluke populations from three different host species (sheep, cattle, and pig) were studied by Valero *et al.* (2001a). No significant differences in the allometric function of UA versus BA between Bolivia liver fluke populations from cattle, sheep and pig were detected. An overlapping of UA versus BA values in liver flukes was obtained from the three Bolivian host species. Function allometry shape was not significantly different between European populations.

Several mammalian species may serve as definitive hosts for *F. hepatica*, but there is considerable variation in susceptibility and induced pathology according to host species. Ross (1968) classified animal hosts of *F. hepatica* in three groups: animals with low, medium or high resistance. Sheep belong to the low resistance group, cattle are in the group of medium resistance species and the pig belongs to the high resistance group. Haroun & Hillyer (1986) reviewed our knowledge on how this resistance affects, to a greater or lesser extent, *F. hepatica* adults. The present study shows that uterine allometry of adult *F. hepatica* with respect to BA follows a pattern which is independent of the host species. In Bolivian pigs it follows the same pattern as in hosts considered normal (such as sheep or cattle) which is of great interest, because in other geographical areas the pig shows substantial natural resistance to infection by *Fasciola* species (Ashizawa *et al.*, 1966; Oshima *et al.*, 1971; Shimizu *et al.*, 1994) or is even considered non-viable as a host for *F. hepatica* (Polyakova-Kr'steva & Gorchilova, 1972). These results lend support to those of Valero & Mas-Coma (2000), who found that there were no differences in the infectivity of the metacercariae between sheep, cattle and pig isolates from the Bolivian Altiplano, and those of Valero *et al.* (2001b) who found that in the Bolivian Altiplano, *F. hepatica* adult development in the pig is similar to that in other host species considered the main reservoirs such as sheep and cattle. All this suggests that pigs may play an important role in fascioliasis transmission in this hyperendemic area and, consequently, must be taken into account when applying control measures.

Conclusions

The aim of this review is to give some indications of the application of computer image analysis and an allometric model concerning the quantification of the different size or shape of *F. hepatica* adults and eggs. The application of these tools makes the calculation of the size of the complex structures of parasite species (such as *F. hepatica*) possible. The allometric model proposed by Valero *et al.* (1996, 1999) (function 2) provides a quantitative analysis of the different factors that can affect the morphological traits of the liver fluke *F. hepatica*, e.g. geographical location or host species. Furthermore, this tool could be used to obtain morphological markers to furnish the base for comparative morphometric studies of fasciolid populations. This tool makes it possible to phenotype the fasciolid adult stage and eggs. A comparison of populations gives rise to statistically significant results based on standardized measurements.

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