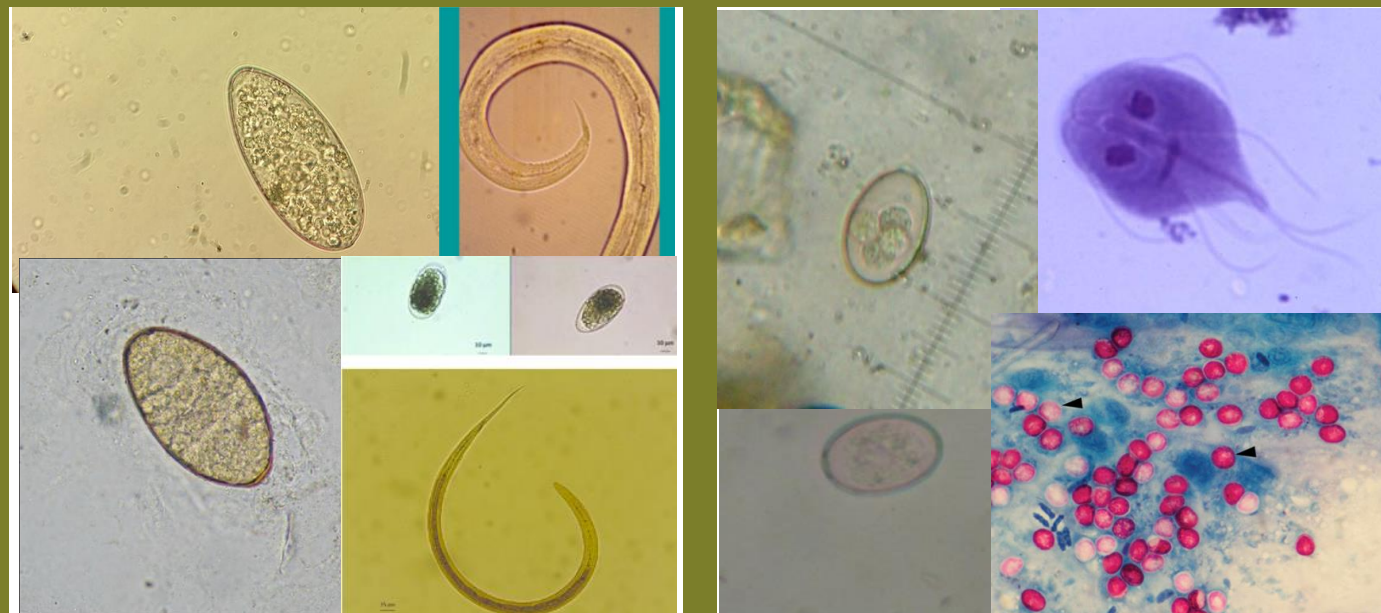




Controllo e gestione dei principali endoparassiti



La gestione di *Giardia* *duodenalis* nei ruminanti



La gestione di *Giardia duodenalis* nei ruminanti

G. duodenalis è un protozoo segnalato in tutto il mondo nei ruminanti. L'infezione può essere causa di diarrea e malassorbimento.

Nella maggior parte dei casi, i giovani animali mostrano tassi di prevalenza più elevati e forme cliniche più frequenti rispetto agli adulti.

Potenziale impatto sulle produzioni per la diminuzione dell'efficienza alimentare e le riduzioni di peso/incremento ponderale.

Epidemiology



Gli assemblaggi identificati nei ruminanti sono: **A**, **B**, E (C, D, F)

L'Assemblaggio E predomina nei ruminanti, ma anche l'assemblaggio **A** è segnalato frequentemente ed è probabilmente più diffuso nei ruminanti di quanto si pensasse in passato.

L'assemblaggio B è meno frequente.

Epidemiology

Bovino



Il picco di prevalenza si osserva **negli animali di età compresa tra 1 e 6 mesi, con una prevalenza più elevata dalle 3 settimane ai 2 mesi di età**. Dai 6 mesi in poi la prevalenza diminuisce. Non sono state riscontrate differenze tra i bovini da carne e quelli da latte.

In uno studio, la prevalenza di *G. duodenalis* nei vitelli di 2-16 settimane di aziende da latte in Germania, Francia, Regno Unito e Italia **(Veneto e Friuli-Venezia Giulia)** è risultata del 45,4%, mentre la prevalenza nelle aziende esaminate dell'89,9%, con differenze sia negli animali (prevalenza fino al 52,6% nei vitelli di 5–8 settimane di età con diminuzione progressiva) che negli allevamenti tra i quattro paesi:

Italia: prevalenza nei vitelli 32%, prevalenza negli allevamenti 88,6%

Elevato rischio di infezione!

PREVALENZA nelle pecore e capre



Fattori gestionali, geografici e climatici e differenze nel numero di animali o di allevamenti esaminati e dei metodi diagnostici utilizzati potrebbero rappresentare i motivi dell'elevata variabilità osservata nei diversi studi.

The animal prevalence (P_A) and farm prevalence (P_F) of *Giardia duodenalis* in other production animals in different countries. The number of animals ($\#_A$) and farms ($\#_F$) is presented along with the diagnostic assay (Diag) used in the study (IFA, Immunofluorescence microscopy; PCR, polymerase chain reaction; or ME, microscopical examination; cELISA, copro-antigen ELISA). -, not known.

Country	Diag	$\#_A$	$\#_F$	P_A	P_F	Reference
<i>Sheep</i>						
Australia	ME	1647	-	9	-	Ryan et al. (2005)
Belgium	IFA	137	10	36	100	Geurden et al. (2008d)
Canada	IFA	89	6	38	100	Olson et al. (1997)
Italy	ME	325	20	1.5	10	Giangaspero et al. (2005)
Spain	IFA	446	38	19	92	Castro-Hermida et al. (2006a)
Spain	IFA	575	68	33	97	Castro-Hermida et al. (2006b)
<i>Goats</i>						
Belgium	IFA	148	10	53	80	Geurden et al. (2008d)
Brazil	ME	105	6	14	66	Bomfim et al. (2005)
Spain	IFA	116	20	20	90	Castro-Hermida et al. (2006a)
Spain	ME	574	-	4	-	Díaz et al. (1996)
Spain	ME/ELISA	315	40	42	95	Ruiz et al. (2008)
<i>Pigs</i>						
Canada	IFA	236	6	9	66	Olson et al. (1997)
Denmark	IFA					Maddox-Hyttel et al. (2006)
Weaners		504	50	38	84	
Piglets		488	50	3	22	
Sows		245	50	4	18	
Norway	IFA	684	100	1.5	10	Hamnes et al. (2007)
Croatia	cELISA	-	38	-	66	Bilic et al. (2006)
<i>Water buffalo</i>						
Italy	cELISA	347	90	18	30	Rinaldi et al. (2007)

Epidemiology



L'incidenza negli allevamenti in cui è stata diagnosticata *G. duodenalis* è del 100% nei bovini e nei caprini e vicina al 100% negli ovini.

Pertanto, la probabilità che i giovani animali di queste aziende si possano infettare è elevatissima.

Epidemiology



L'escrezione massima di cisti può arrivare fino a 10^6 cisti per grammo di feci negli animali giovani, a causa del lento sviluppo dell'immunità adattativa da parte dell'ospite.

Gli animali giovani devono quindi essere considerati la principale fonte di infezione per altri ospiti sensibili.

Lo sviluppo dell'immunità adattativa si traduce in una escrezione intermittente di cisti e in una ridotta escrezione di cisti.

Gli animali trattati sono recettivi a una nuova infezione subito dopo il trattamento.

Inoltre, in pecore, capre e bovini è stato osservato un aumento dell'escrezione di cisti nel periparto.

Epidemiology



L'elevata escrezione di cisti da parte degli animali infetti e la bassa dose necessaria per l'infezione può facilitare la trasmissione.

Le cisti di *Giardia* sono immediatamente infettanti quando eliminate con le feci.

Sono anche molto resistenti e in grado di sopravvivere per diverse settimane nell'ambiente, determinando un graduale aumento della contaminazione ambientale.

Epidemiology



L'infezione può diffondersi direttamente (**animale-animale, zoonotico (ad esempio, allevatori, veterinari e operatori)**), oppure indirettamente, tramite ambienti, alimenti o acqua contaminati da cisti.

È noto che le infezioni si diffondono nelle stalle o nelle gabbie a causa del successivo utilizzo di queste strutture senza una adeguata disinfezione.

I recinti di maternità sono stati considerati una fonte indiretta di infezione.

L'infezione può anche diffondersi attraverso utensili o persone che si prendono cura degli animali.

Epidemiology



A causa della concentrazione di animali in grado di eliminare un numero elevato di cisti infettanti e della continua introduzione di animali sensibili, **i sistemi di gestione intensiva** con ricollocazione degli animali giovani in strutture di stabulazione dedicate favoriscono la trasmissione dell'infezione.

Gli animali allevati al chiuso hanno una probabilità di acquisire l'infezione superiore di tre volte rispetto a quelli allevati all'aperto.

La gestione di *Giardia duodenalis* nei ruminanti

Anche la gravità dell'infezione dipende in gran parte dal sistema di allevamento, con un impatto maggiore sulla salute degli animali e sulle produzioni **negli allevamenti intensivi o con stabulazione al chiuso rispetto ai sistemi estensivi.**

Epidemiologia



Fattori di rischio

Numerosi fattori gestionali sono stati evidenziati come fattori di rischio, tra cui:

1. Misure igieniche ambientali inadeguate:

La **pulizia e la disinfezione** sono fondamentali per prevenire il ripetersi di infezioni e di forme cliniche dopo il trattamento degli animali e per ridurre al minimo la trasmissione dell'infezione attraverso ambienti contaminati.

Le seguenti procedure sono significativamente correlate alla diminuzione delle probabilità di infezione:

1. pulizia regolare dei recinti di parto
2. Pulizia e disinfezioni delle strutture utilizzate per i vitelli/agnelli/capretti
3. Periodo di vuoto tra i diversi gruppi di animali
4. Rimozione fecale prima di introdurre un nuovo gruppo di animali
5. Pulizia quotidiana delle stalle con pavimenti in cemento

Controllo

Per ridurre al minimo il rischio di reinfezione dopo il trattamento, è necessario utilizzare un programma di controllo integrato che combini il trattamento e la pulizia-disinfezione dell'ambiente alla fine del periodo di trattamento.

L'efficacia di una combinazione di trattamento animale e pulizia e disinfezione ambientale o il trasferimento degli animali trattati in altri ambienti non contaminati, determina una riduzione significativa sia del numero di animali che eliminano cisti che del numero di cisti escrete.

Controllo

La corretta gestione può contribuire a ridurre il tasso di infezione.

Queste misure gestionali includono:

1. Tenere bassa la densità degli animali
2. Rinnovo frequente della lettiera combinato con pulizia e disinfezione regolari delle stalle
3. Una pavimentazione solida favorisce la trasmissione.

Epidemiology



Fattori di rischio

2. Il contatto con la madre

Vitelli, agnelli e capretti a contatto con la madre hanno maggiori probabilità di infezione rispetto ad animali separati dalla madre. Negli allevamenti positivi, le madri devono essere considerate una potenziale fonte di infezione, probabilmente a causa dell'aumento dell'escrezione di cisti nel periodo del parto.

L'effetto benefico di ridurre al minimo il contatto diretto tra gli animali era stato precedentemente suggerito, separando il vitello dalla madre immediatamente dopo la nascita o impedendo il contatto tra diverse coppie vacca-vitello.

Controllo

Adeguata gestione del colostro

Può aiutare a prevenire l'insorgenza di forme cliniche e, in misura minore, delle infezioni, attraverso il trasferimento passivo di immunoglobuline specifiche, ma anche attraverso componenti aggiuntivi, come leucociti, citochine, componenti bioattivi, fattori di crescita e un'alta concentrazione di nutrienti, che aiutano per far fronte alle infezioni nelle prime fasi della vita.

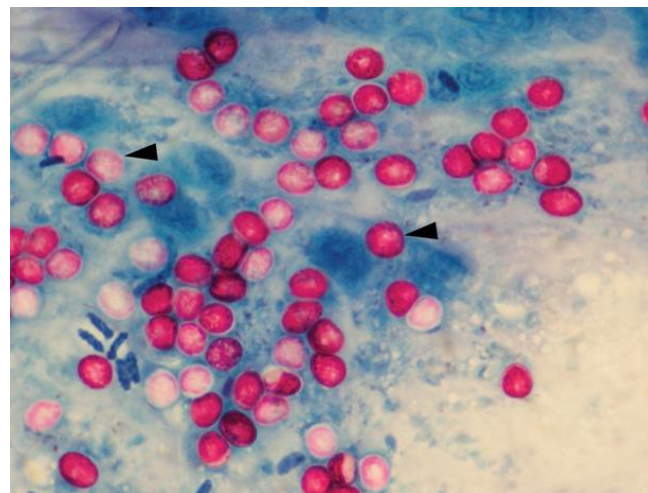
Parasite characteristics of *G. duodenalis* in production animals, the epidemiological consequence and the appropriate preventive measures.



Parasite characteristic	Epidemiological consequence	Preventive measure
High excretion of cysts in the faeces, which are immediately infective	Infection pressure can increase in a short period of time	-Avoid crowding -Isolation of excreting calves -Hygiene*
Cysts are resistant to environmental conditions	Excreted cysts can survive for several weeks in the environment	-Hygiene*
Cysts are resistant to chemical disinfection	Disinfection with common disinfectants is not reliable	Products based on ammonia, chlorine dioxide, hydrogen dioxide or ozone
Cysts are sensitive to heat and desiccation	Infection is mostly seen in stables	-Disinfection using steam -Avoid crowding -Hygiene*

- Thorough cleaning, preferably using a high pressure device.
- Additional vacancy during a prolonged period.
- Or alternate use of stable compartments.

* Hygiene—frequent removal of faeces.



La gestione della Criptosporidiosi nei ruminanti

Infezione particolarmente grave nei ruminanti neonati



Contents lists available at SciVerse ScienceDirect

Small Ruminant Research

journal homepage: www.elsevier.com/locate/smallrumres



Cryptosporidiosis in small ruminants[☆]

C. Paraud*, C. Chartier

ANSES, Laboratoire de Niort, 60 rue de Pied de Fond, 79012 Niort, France

ARTICLE INFO

Article history:
Available online 16 November 2011

Keywords:
Cryptosporidium
Small ruminants
Goat
Sheep
Zoonosis

ABSTRACT

Cryptosporidiosis is an infection caused by protozoan parasites belonging to the genus *Cryptosporidium* which is responsible for a potentially severe disease in new-born ruminants. This infection is highly prevalent in small ruminants throughout the world, especially in pre-weaned animals. The clinical expression is different between goat kids and lambs, the infection being generally more severe in the former. Molecular data demonstrate geographical variations in the species of *Cryptosporidium* infecting small ruminants. They also support the possibility of transmission of zoonotic species from these hosts to humans. Studies are still needed on molecular epidemiology, especially in goats, and on ways to control infection.



Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar



Cryptosporidium and *Giardia* associated with reduced lamb carcass productivity

Joshua P.A. Sweeny*, U.M. Ryan, I.D. Robertson, C. Jacobson

School of Veterinary and Biomedical Sciences, Murdoch University, Perth, Western Australia, 6150, Australia

ARTICLE INFO

Article history:
Received 9 February 2011
Received in revised form 24 May 2011
Accepted 30 May 2011

Keywords:
Sheep
Cryptosporidium
Giardia
Production
Carcass weight
Faecal consistency

ABSTRACT

On two extensive sheep farms in southern Western Australia, 111 (Farm A) and 124 (Farm B) female crossbred lambs (2–6 weeks old) were randomly selected and individually identified using ear tags (a numbered tag and radio-frequency tag) at marking. On five separate occasions, faecal samples were collected and live weight, body condition score (BCS), faecal consistency score (FCS), breech fleece faecal soiling score and faecal dry matter percentage (DM%) were recorded. Lamb hot carcass weight (HCW) and dressing percentage were measured at slaughter. Faecal samples were screened by PCR for *Cryptosporidium* (18S rRNA, actin and 60 kDa glycoprotein [gp60] loci), *Giardia duodenalis* (glutamate dehydrogenase [gdh] and triosephosphate isomerase [tpi]) and *Campylobacter jejuni* (16S rRNA). Observation of *Elmeria* oocysts and faecal worm egg counts (WECs) were performed using a modified McMaster technique. The WECs were adjusted for FCS for analyses. Faecal samples were screened for patent strongylid infections using PCR (specifically ITS-2 nuclear ribosomal DNA for *Teladorsagia circumcincta*, *Trichostrongylus* spp. and *Haemonchus contortus*). Lambs positive for *Cryptosporidium* at least once had lighter HCWs by 1.25 kg (6.6%) ($P=0.029$) and 1.46 kg (9.7%) ($P<0.001$) compared to lambs never positive for *Cryptosporidium* for Farms A and B respectively. Similarly, dressing percentages were 1.7% ($P=0.022$) and 1.9% ($P<0.001$) lower in *Cryptosporidium*-positive lambs on Farms A and B respectively. Lambs positive for *Giardia* at least once had 0.69 kg ($P<0.001$) lighter HCWs and 1.7% ($P<0.001$) lower dressing percentages compared to lambs never positive for *Giardia* on Farm B only. *Cryptosporidium*-positive lambs at the second sampling were 4.72 ($P=0.010$) and 3.84 ($P=0.002$) times more likely to have non-pelleted faeces compared to *Cryptosporidium*-negative lambs for Farms A and B respectively. Breech fleece faecal soiling scores of *Cryptosporidium*-positive lambs were 3.36 ($P=0.026$) and 2.96 ($P=0.047$) times more likely to be moderate to severe (scores 3–5), compared to negative lambs at the second sampling for Farms A and B respectively. Live weight, growth rate and BCS were inconsistently associated with protozoa detection across different samplings and farms. Adjusted WEC was correlated positively with FCS and negatively with faecal DM%, differing between sampling occasions and farms. *Campylobacter jejuni* prevalence was very low (<1%). Adjusted WEC were not correlated with carcass attributes, growth rates or live weights. This study is the first to quantify productivity consequences of naturally acquired protozoa infections in lambs managed under extensive farming conditions.

© 2011 Elsevier B.V. All rights reserved.

Table 1. Valid species of *Cryptosporidium*. Many of these earlier species were originally described based on morphological criteria but have subsequently been validated using molecular data

Species name	Author(s)	Type hosts	Major host	Reports in humans
<i>C. muris</i>	Tyzzer (1907, 1910)	Tame mice	Rodents	Numerous reports (cf. Feng <i>et al.</i> 2011b)
<i>C. wrairi</i>	Vetterling <i>et al.</i> (1971)	<i>Cavia porcellus</i> (Guinea pig)	Guinea pigs	None reported
<i>C. felis</i>	Isekü (1979)	<i>Felis catus</i> (Cat)		Many reports (cf. Lucio-Forster <i>et al.</i> 2010)
<i>C. serpentis</i>	Levine (1980)	<i>Elaphe guttata</i> , <i>E. subocularis</i> , <i>Sanzinia madagascarensis</i> (Snakes)	Snakes and lizards	None reported
<i>C. meleagridis</i>	Slavin (1955)	<i>Meleagris gallopavo</i> (Turkey)	Birds and humans	Commonly reported in humans
<i>C. parvum</i>	Re: Upton and Current (1985) Tyzzer (1912) ^a	<i>Bos taurus</i> (Cattle)	Ruminants	Commonly reported in humans
<i>C. baileyi</i>	Current <i>et al.</i> (1986)	<i>Gallus gallus</i> (Chicken)	Birds	None reported
<i>C. varanii</i>	Pavlásek <i>et al.</i> (1995)	<i>Varamus prasinus</i> (Emerald Monitor)	Lizards	None reported
<i>C. andersoni</i>	Lindsay <i>et al.</i> (2000)	<i>Bos taurus</i> (Cattle)	Cattle	Leoni <i>et al.</i> (2006); Morse <i>et al.</i> (2007); Waldron <i>et al.</i> (2011); Agholi <i>et al.</i> (2013); Liu <i>et al.</i> (2014)
<i>C. canis</i>	Fayer <i>et al.</i> (2001)	<i>Canis familiaris</i> (Dog)	Dogs	Many reports (cf. Lucio-Forster <i>et al.</i> 2010)
<i>C. molnari</i>	Alvarez-Pellitero and Sitja-Bobadilla (2002)	<i>Sparus aurata</i> and <i>Dicentrarchus labrax</i> (Fish)	Fish	None reported
<i>C. hominis</i>	Morgan-Ryan <i>et al.</i> (2002)	<i>Homo sapiens</i> (Human)	Humans	Most common species in humans
<i>C. galli</i>	Re: Ryan <i>et al.</i> (2003c) Pavlásek (1999) ^a	Spermestidae, Frangillidae, <i>Gallus gallus</i> , <i>Tetrao urogallus</i> , <i>Pinicola enucleator</i> (Birds)	Birds	None reported
<i>C. suis</i>	Ryan <i>et al.</i> (2004)	<i>Sus scrofa</i> (Pig)	Pigs	Xiao <i>et al.</i> (2002a); Leoni <i>et al.</i> (2006); Cama <i>et al.</i> (2007); Wang <i>et al.</i> (2013)
<i>C. bovis</i>	Fayer <i>et al.</i> (2005)	<i>Bos taurus</i> (Cattle)	Cattle	Khan <i>et al.</i> (2010); Ng <i>et al.</i> (2012); Helmy <i>et al.</i> (2013)
<i>C. fayeri</i>	Ryan <i>et al.</i> (2008)	<i>Macropus rufus</i> (Kangaroo)	Marsupials	Waldron <i>et al.</i> (2010)
<i>C. fragile</i>	Jirku <i>et al.</i> (2008)	<i>Duttaphrym melanostictus</i> (Toad)	Toads	None reported
<i>C. macropodum</i>	Power and Ryan (2008)	<i>Macropus giganteus</i> (Kangaroo)	Marsupial	None reported
<i>C. ryanae</i>	Fayer <i>et al.</i> (2008)	<i>Bos taurus</i> (Cattle)	Cattle	None reported
<i>C. xiaoi</i>	Fayer <i>et al.</i> (2010)	<i>Ovis aries</i> (Sheep)	Sheep and goats	Adamu <i>et al.</i> (2014)
<i>C. ubiquitum</i>	Fayer <i>et al.</i> (2010)	<i>Bos taurus</i> (Cattle)	Ruminants, rodents, primates	Commonly reported (cf. Fayer <i>et al.</i> 2010; Elwin <i>et al.</i> 2012a)
<i>C. cuniculus</i>	Re: Robinson <i>et al.</i> (2010) Inman and Takeuchi (1979) ^a	<i>Oryctolagus cuniculus</i> (European rabbit)	Rabbits	Chalmers <i>et al.</i> (2009b); Anon (2010); Molloy <i>et al.</i> (2010); Chalmers <i>et al.</i> (2011a)
<i>C. tyzzeri</i>	Re: Ren <i>et al.</i> (2012) Tyzzer (1912) (<i>C. parvum</i>) ^a	<i>Mus musculus</i> (Mouse)	Rodents	Raskova <i>et al.</i> (2013)
<i>C. viatorum</i>	Elwin <i>et al.</i> (2012b)	<i>Homo sapiens</i> (Human)	Humans	Elwin <i>et al.</i> (2012b); Insulander <i>et al.</i> (2013)
<i>C. scrofarum</i>	Kváč <i>et al.</i> (2013b)	<i>Sus scrofa</i> (Pig)	Pigs	Kváč <i>et al.</i> (2009a); Kváč <i>et al.</i> (2009b)
<i>C. erinacei</i>	Kváč <i>et al.</i> (2014b)	European hedgehog (<i>Erinaceus europaeus</i>)	Hedgehogs and horses	Kváč <i>et al.</i> (2014a)

Re, redescription.

^a Initial description

Specie e genotipi zoonotici

Numerose specie e genotipi di *Cryptosporidium* sono stati riportati nell'uomo, inclusi *C. hominis*, *C. parvum*, *C. meleagridis*, *C. felis*, *C. canis*, *Cryptosporidium cuniculus*, *C. ubiquitum*, *Cryptosporidium viatorum*, *C. muris*, *Cryptosporidium suis*, *Cryptosporidium fayeri*, *C. andersoni*, *Cryptosporidium bovis*, *Cryptosporidium scrofarum*, *Cryptosporidium tyzzeri*, *Cryptosporidium erinacei* e *Cryptosporidium* genotipo cavallo

Cryptosporidium

Caratteristiche

- (i) la localizzazione all'interno della cellula ospite, dove gli stadi dello sviluppo endogeno sono confinati alle superfici apicali della cellula ospite (intracellulare, ma extracitoplasmatica);
- (ii) l'adesione del parassita alla cellula ospite, in cui alla base del vacuolo parassito si forma una struttura multi-membranosa o **feeder organelle** per facilitare l'assorbimento di sostanze nutritive dalla cellula ospite;
- (iii) la presenza di due tipi di oocisti, a parete spessa e a parete sottile, quest'ultima è responsabile di autoreinfezioni nell'ospite infetto;
- (iv) le dimensioni ridotte dell'oocisti che sono prive di sporocisti, micropili e granuli polari;
- (v) l'insensibilità a tutti gli agenti anti-coccidi testati fino ad oggi;
- (vi) **Notevole resistenza oocisti nell'ambiente** (3 mesi a 15-20 °C e più di 1 anno a 4-6°C) ed ai comuni disinfettanti (sensibili solo nei confronti dell'ammoniaca a 5-50%, formalina al 10% e perossido di idrogeno al 3%).

Opinion

Life without a Host Cell:
What is *Cryptosporidium*?Peta L. Clode,^{1,*} Wan H. Koh,² and R.C. Andrew ThompTrends in Parasitology 2014, 14:201
http://www.trendsparasitology.com/content/14/1/201

RESEARCH ARTICLE

Open Access

Extracellular excystation and development of
Cryptosporidium: tracing the fate of oocysts within
Pseudomonas aquatic biofilm systemsWan Koh^{1,2}, Andrew Thompson², Hanna Edwards², Paul Morris² and Peta L. Clode¹

Abstract

Background: Aquatic biofilms often serve as environmental reservoirs for microorganisms and provide them with a nutrient-rich growth environment under harsh conditions. With regard to *Cryptosporidium*, biofilms can serve as environmental reservoirs for oocysts, but may also support the growth of additional *Cryptosporidium* stages.**Results:** Here we used confocal laser scanning microscopy, scanning electron microscopy (SEM), and flow cytometry to identify and describe various *Cryptosporidium* developmental stages present within aquatic biofilm systems, and to directly compare these to stages produced in cell culture. We also show that *Cryptosporidium* has the ability to form a parasitological, viable independently, in a host-free biofilm environment, potentially allowing them to complete an extracellular life cycle. Correlative data from confocal and SEM imaging of the same cells confirmed that the observed developmental stages (including trophozoites, meronts, and merozoites) were *Cryptosporidium*. These microscopy observations were further supported by flow cytometric analysis, where excysted oocyst populations were detected in 1, 3 and 4-day-old *Cryptosporidium*-exposed biofilms, but not in biofilm-free controls.**Conclusions:** These observations not only highlight the risk that aquatic biofilms pose in regards to *Cryptosporidium* outbreaks from water distribution systems, but further indicate that even single biofilms are able to stimulate oocyst excystation and support the extracellular multiplication and development of *Cryptosporidium* within aquatic environments.**Keywords:** *Cryptosporidium*, Biofilms, Extracellular multiplication, Excystation, Confocal microscope, Scanning electron microscopy, Flow cytometryWan et al. *Trends in Parasitology* 2014, **14**:201
http://www.trendsparasitology.com/content/14/1/201

RESEARCH

Open Access

Multiplication of the waterborne pathogen
Cryptosporidium parvum in an aquatic biofilm
systemWan Koh^{1,2}, Peta L. Clode¹, Paul Morris² and R.C. Andrew Thompson²

Abstract

Background: In natural aquatic environments biofilms are known to act as environmental reservoirs for *Cryptosporidium parvum* oocysts. However, the fate of these oocysts within biofilms has yet to be determined.**Methods:** This study aimed to identify if biofilms have the ability to support the multiplication of *Cryptosporidium* by measuring the change in parasite number over time using quantitative polymerase chain reaction (qPCR) and detecting the possible extracellular developmental stages using a combination of confocal microscopy and immunolabeling techniques. *Pseudomonas aeruginosa* biofilm flow cell systems were established and *C. parvum* oocysts were constantly supplied over a 16 day period.**Results:** A significant ($P < 0.001$) increase in *Cryptosporidium* was detected as the biofilm matured, with the total number of *C. parvum* multiplying 2–3 fold during this period. With this, various *Cryptosporidium* developmental stages (trophozoites, trophozoites, type 1 and 2 meronts) were identified from the biofilm.**Conclusions:** This is the first study demonstrating that biofilms not only serve as an environmental reservoir for oocysts, but are also capable of supporting the multiplication of *Cryptosporidium* over time in an aquatic environment.**Keywords:** *Cryptosporidium*, Biofilms, Extracellular multiplication, Water, qPCR, Fluorescence

**Dimostrata la moltiplicazione
di *Cryptosporidium* nei
biofilm acquatici (senza
necessità dell'ospite)**

Trasmissione

- **Trasmissione oro-fecale:**

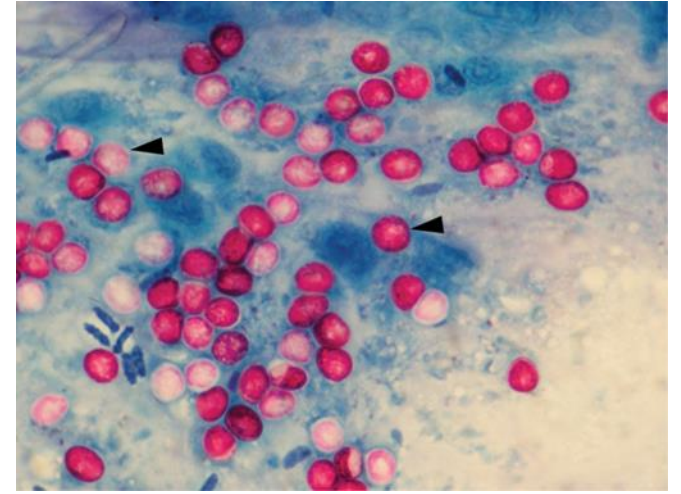
1. Acqua

2. Alimenti

- **Trasmissione diretta**

- **Animale-Animale e Animale-Uomo**

Ruolo delle madri nei ruminanti per l'emissione di oocisti nell'ultimo periodo di gravidanza-primo periodo di lattazione



**La dose infettante è molto bassa
Negli agnelli la dose minima può corrispondere
a una singola oocisti e la dose infettante media
è di circa 5 oocisti.**

Epidemiologia criptosporidiosi ruminanti Bovini

Prevalenza

- **Italia: dal 3,4 al 35,6% degli animali negli allevamenti esaminati**
- **Toscana 20% dei vitelli (di età inferiore ai 30gg) positivi su 157 esaminati**
- **In uno studio condotto in Italia è stato notato che la pratica di separare il vitello dalla madre subito dopo la nascita rappresenta un fattore di rischio per una maggior probabilità per il vitello di sviluppare una criptosporidiosi clinica.**



Bovini

La criptosporidiosi bovina è stata segnalata in tutto il mondo indipendentemente dal sistema di allevamento.

I bovini possono essere colpiti da quattro specie di *Cryptosporidium*: *C. parvum*, *C. bovis*, *C. ryanae* e *C. andersoni*.

Nei paesi occidentali *C. parvum* predomina nei vitelli pre-svezzamento.

Negli allevamenti endemici i tassi di morbidità raggiungono il 100% ma la mortalità è bassa.

I vitelli sembrano acquisire l'infezione subito dopo la nascita con i più alti tassi di infezione riportati nei vitelli da 1 a 3 settimane di età.



Bovini

C. parvum indubbiamente riconosciuto come un agente primario di diarrea nei vitelli di meno di 3 settimane di età.

Le differenze nella gravità della criptosporidiosi potrebbero essere correlate alla presenza di infezioni concomitanti con altri enteropatogeni, a isolati che mostrano differenze di virulenza e nella produzione di oocisti, oltre che a numerosi fattori gestionali.



Numerose le epidemie riportate tra studenti di Veterinaria e tra il personale a contatto con bovini nelle stalle e nei laboratori in diversi paesi del mondo



Epidemiologia criptosporidiosi ruminanti Pecore

La prevalenza negli ovini può variare da <5 a> 70%

Almeno otto specie di *Cryptosporidium* sono state identificate negli ovini, tra cui *C. parvum*, *C. hominis*, *C. andersoni*, *C. suis*, *C. xiaoi*, *C. fayeri*, *C. ubiquitum* e *C. scrofarum*

C. parvum è la specie predominante in Europa ed è la specie più frequentemente causa di diarrea negli agnelli, mentre *C. xiaoi* e *C. ubiquitum* sono più spesso riscontrati negli agnelli sani

Criptosporidiosi ruminanti: Pecore

***Cryptosporidium* è un agente eziologico primario di diarrea e un importante enteropatogeno associato a diarrea neonatale negli agnelli.**

Le oocisti di *Cryptosporidium* si osservano principalmente negli animali molto giovani (1-3 settimane di età), con una ridotta incidenza negli animali più anziani.

Le epidemie di *Cryptosporidium* si osservano di solito negli agnelli molto giovani, entro le prime 2 settimane di vita, e sebbene la mortalità sia generalmente bassa, aumenta con infezioni intercorrenti o altri fattori concomitanti, come carenze nutrizionali e errori di management.

Il rischio di epidemie da *Cryptosporidium* è infatti maggiore negli allevamenti affollati, probabilmente a causa delle cattive condizioni igieniche e di errori di gestione.



Criptosporidiosi ruminanti Pecore

L'infezione può essere responsabile di perdite economiche ingenti che riflettono non solo la mortalità, ma anche i ridotti tassi di crescita dopo la guarigione.

Adulti e agnelli sani possono liberare le oocisti, fungendo da fonte di infezione per altri animali, possibilmente mantenendo la criptosporidiosi all'interno di un gregge tra i periodi di parto.

Nelle pecore è stato osservato un aumento di escrezione di oocisti nel periodo del parto.



Epidemiologia criptosporidiosi ruminanti:

Capra



Nelle capre sono riportate le specie *C. parvum*, *C. hominis*, *C. ubiquitum* e *C. xiaoi*

Nell'allevamento caprino, la criptosporidiosi è considerata una malattia economicamente molto importante e caratterizzata da diarrea e mortalità nei capretti.

In alcuni allevamenti, la morbilità può raggiungere il 100% e la mortalità il 50%.

Una associazione significativa con la diarrea è stata riscontrata nei capretti infetti da *Cryptosporidium*.

Casi di epidemie ripetute nelle capre, con anoressia, prostrazione e diarrea con feci pastose e giallastre.

Epidemiologia criptosporidiosi ruminanti

Capre

Nei capretti con infezione naturale, l'escrezione di oocisti di *Cryptosporidium* inizia a 4 giorni di età con un picco a 7 giorni di età e un calo dopo 3 settimane.

Una assunzione insufficiente di colostro e la contaminazione delle aree destinate ai capretti è frequentemente associata all'infezione da *Cryptosporidium*.

Un aumento dell'escrezione di oocisti nel periparto e lunghi periodi di eliminazione di oocisti da parte di animali asintomatici potrebbero servire come fonti di infezione per i capretti



Controllo:

ANIMALI:

- parti distocici nei bovini

- Stabulare madre e figli separati da altri vitelli

- Stabulare in box singoli vitelli e capretti per almeno 2-3 sett. o separarli per gruppi di età

Si riduce l'esposizione al patogeno nella fase più critica

Nascita di animali ipossici con acidosi metabolica
Stress per la madre = scarsa produzione di colostro

Altri vitelli possono assumere colostro destinato al figlio

Scarsa assunzione di colostro
scarsa
protezione vs *Cryptosporidium*

Controllo

- **Separare eventuali malati dai sani quanto prima**
- **Se il patogeno entra in allevamento attenzione all'uomo per la trasmissione**

Controllo

Controllo ambientale

- **Rinnovare frequentemente la lettiera**
- **A fine ciclo di allevamento, pulizia locali ed attrezzature e poi vuoto sanitario**
- **Pulizia accurata con getto di vapore oppure con acqua molto calda ad alta pressione seguita da asciugatura.**
- **Disinfezione con disinfettanti a base di perossido di idrogeno**

Le oocisti sono sensibili solo a temperature estreme (sotto i $-20\text{ }^{\circ}\text{C}$ e oltre i 60°C) e all'essiccazione.

Controllo

- Il rischio di epidemie di criptosporidiosi è più elevato in caso di sovraffollamento, scarse condizioni igieniche ed errori di gestione.
- Alcune procedure gestionali sono considerate fattori di rischio elevato per la criptosporidiosi clinica negli agnelli e nei capretti. Tra queste sono incluse:
 - la separazione precoce degli agnelli dalle madri
 - la mancata assunzione di colostro prodotto dalle madri
 - il sovraffollamento degli spazi destinati agli agnelli
 - le malattie concomitanti e altri fattori stressanti.

Cryptosporidium infections in sheep farms from Italy

G. Dessì¹ • C. Tamponi¹ • A. Varcasia¹  • G. Sanna¹ • A. P. Pipia¹ • S. Carta¹ • F. Salis² • P. Díaz¹ • A. Scala¹

Received: 12 August 2020 / Accepted: 25 October 2020

© The Author(s) 2020

Abstract

Cryptosporidiosis is recognized as being a significant cause of gastrointestinal illness due to its wide range of vertebrate hosts, including humans. Infection with *Cryptosporidium* spp. is especially common in young domestic ruminants (calves, lambs and goat kids) and has been associated with economic losses worldwide. In contrast to cattle, to date, detailed studies on *Cryptosporidium* infections in sheep from Europe are still limited; thus, their importance as reservoirs of *Cryptosporidium* species with implications on animal and public health still needs to be clarified. This study evaluates the prevalence and zoonotic potential of *Cryptosporidium* spp. in sheep farms in Italy. A total of 915 individual faecal samples divided into three different animal categories were collected from 61 sheep farms. Each sample was examined by microscopy of faecal smears stained by modified Ziehl-Neelsen and by biomolecular techniques. *Cryptosporidium* oocysts were detected in 10.1% of the animals examined and in 34.4% of the farms. The prevalence of *Cryptosporidium* spp. was significantly higher ($\chi^2 = 51.854$; $P < 0.001$) in diarrhoeic samples than in pasty or normal faeces. Genotype analyses showed the presence of two *Cryptosporidium* species: *C. parvum* and *C. ubiquitum*. Subtyping analysis of *C. parvum* isolates revealed the presence of subtypes IIa15G2R1 and IIa20G1 and of subtype XIIa for *C. ubiquitum*. These findings have public health implications since both *Cryptosporidium* species identified are considered zoonotic, and *C. parvum* is the second-most common *Cryptosporidium* species infecting humans. Our data reveal that lambs, especially those excreting diarrhoeic faeces, may be important reservoirs of *Cryptosporidium*. We also highlight the need to establish adequate control and monitoring programmes for the control of this infection in sheep farms primarily through coprological monitoring.



Promising Anti-Protozoan Activities of Propolis (Bee Glue) as Natural Product: A Review

Shabnam Asfaram^{1,2} · Mahdi Fakhar² · Masoud Keighobadi² · Javad Akhtari³

Received: 12 March 2020 / Accepted: 9 July 2020
© Witold Stefański Institute of Parasitology, Polish Academy of Sciences 2020

Abstract

Purpose Propolis (bee glue) is a resinous mixture of different plant exudates that possesses a wide range of biological and antimicrobial activities and has been used as a food supplement and in complementary medicine for centuries. Some researchers have proposed that propolis could be a potential curative compound against microbial agents such as protozoan parasitic infections by different and occasionally unknown mechanisms due to the immunoregulatory function and antioxidant capacity of this natural product.

Methods In this review, we concentrate on in vitro and in vivo anti-protozoan activities of propolis extracts/fractions in the published literature.

Results In *Leishmania*, propolis inhibits the proliferation of promastigotes and produces an anti-inflammatory effect via the inhibition of nitric oxide (NO) production. In addition, it increases macrophage activation, TLR-2, TNF- α , IL-4, IL-17 production, and downregulation of IL-12. In *Plasmodium* and *Trypanosoma*, propolis inhibits the parasitemia, improving anemia and increasing the IFN- γ , TNF- α , and GM-CSF cytokines levels, most likely due to its strong immunomodulatory activity. Moreover, propolis extract arrests proliferation of *T. cruzi*, because it has aromatic acids and flavonoids. In toxoplasmosis, propolis increases the specific IgM and IgG titers via decreasing the serum IFN- γ , IL-1, and IL-6 cytokines levels in the rats infected with *T. gondii*. In *Cryptosporidium* and *Giardia*, it decreases oocysts shedding due to phytochemical constituents, particularly phenolic compounds, and increases the number of goblet cells. Propolis inhibits the growth of *Blastocystis*, possibly by apoptotic mechanisms like metronidazole. Unfortunately, the mechanism action of propolis' anti-*Trichomonas* and anti-*Acanthamoeba* is not well-known yet.

Conclusion Reviewing the related literature could highlight promising antimicrobial activities of propolis against intracellular and extracellular protozoan parasites; this could shed light on the exploration of more effective drugs for the treatment of protozoan parasitic infections in the near future.

Keywords Propolis · Bee glue · Natural product · Anti-protozoan activities · Immunoregulatory



Short communication: Effect of pomegranate-residue supplement on *Cryptosporidium parvum* oocyst shedding in neonatal calves

S. Weyl-Feinstein,*†¹ A. Markovics,‡ H. Eitam,*† A. Orlov,* M. Yishay,* R. Agmon,* J. Miron,§ I. Izhaki,† and A. Shabtay*

*Department of Ruminant Science, Institute of Animal Science, Neve Ya'ar Research Center, Agricultural Research Organization, PO Box 1021, Ramat Yishay 30095, Israel

†Department of Evolutionary and Environmental Biology, Faculty of Sciences, University of Haifa, 31905 Haifa, Israel

‡Department of Parasitology, Kimron Veterinary Institute, PO Box 12, Bet-Dagan 50250, Israel

§Department of Ruminant Science, Institute of Animal Science, Agricultural Research Organization, PO Box 6, 50250, Bet-Dagan, Israel

ABSTRACT

Cryptosporidium parvum is considered one of the most common enteropathogens, responsible for the high incidence of diarrhea and deleterious implications on immunity and health in neonatal calves. The pomegranate is well known for its health-promoting properties. Two experiments were designed to test the antiparasitical and antidiarrheal effects of concentrated pomegranate extract (CPE) supplement in milk in neonatal Holstein calves. Forty-one calves were randomly divided into control ($n = 20$) and treatment ($n = 21$) groups. For the first experiment, the treatment group was supplemented with 3.75% CPE in the daily milk ration, between 3 and 14 d of age, whereas the control group received only milk. Fecal samples were collected between d 5 and 13 to quantify *Cryptosporidium* oocysts, and the duration and intensity of diarrhea were evaluated. Reduced fecal oocyst count and diarrhea intensity and duration were revealed in the 3.75% CPE calves. No difference was noted in average daily gain between groups. In a second experiment, which was designed to test the effect of a lower CPE concentration (0.6% of daily milk allocation), no effects on fecal oocyst count and average daily gain were observed. However, compared with control, the lower CPE group was characterized by a shorter duration of diarrhea and higher weight gain among males at 14 d of age. These results suggest that the CPE supplement-to-calf milk ratio may potentially alleviate intestinal morbidity caused by *Cryptosporidium*.

Key words: *Cryptosporidium parvum*, cryptosporidiosis, neonatal calves, pomegranate extract.

Short Communication

The apicomplexan protozoan parasite *Cryptosporidium parvum* is considered one of the most common enteropathogens affecting neonatal calves (Geurden et al., 2006; Santín et al., 2008); it causes enteric infection and diarrhea in many species of mammals (O'Donoghue, 1995) and is a well-known cause of diarrhea in humans and in calves younger than 1 mo (Silverlås et al., 2009). Worldwide studies indicate that within herds its prevalence ranges from 53 to 100% (Lassen et al., 2009; Silverlås et al., 2010). Pathogenesis results from a fecal-oral route transmission preceded by sporozoites encysting the oocysts, whereupon the parasite invades the epithelium resulting in its destruction, villus atrophy, and microvillus shortening (de Graaf et al., 1999); consequently, diarrhea is a typical outcome.

Infection starts as early as 3 d of age (Snodgrass et al., 1980; Xiao and Herd, 1994), peaks at 6 to 15 d of age (Quílez et al., 1996), followed by a gradual reduction of oocyst shedding (Fayer et al., 1998) during the preweaned period (Xiao, 2010). Oocyst numbers can reach the magnitude of 10^6 to 10^7 /g of feces (Current, 1985); however, even small numbers of oocysts are sufficient to infect the gut (De Waele et al., 2010), causing diarrhea, often complicated by metabolic acidosis and electrolyte imbalances (Kasari, 1999), that may lead to dehydration and death (Chen et al., 2003).

Effective therapy for cryptosporidiosis is limited (Cevallos et al., 2000), so the main mode of action remains preventive hygiene management. *Cryptosporidium parvum* oocysts are highly resistant to environ-

S. Perrucci · G. Fichi · C. Buggiani ·
G. Rossi · G. Flamini

Efficacy of mangiferin against *Cryptosporidium parvum* in a neonatal mouse model

Received: 8 February 2006 / Accepted: 16 February 2006 / Published online: 18 March 2006
© Springer-Verlag 2006

Abstract The inhibitory activity of mangiferin (50 mg/kg/die and 100 mg/kg/die) on *Cryptosporidium parvum* was evaluated in a neonatal mouse model and its activity was compared with that of paromomycin (100 mg/kg/die). At 4 days of age, neonatal Swiss conventional outbred mice were experimentally infected by oral administration of 10^6 oocysts/animal of *C. parvum* and treated orally for 10 consecutive days, starting 7 days after the experimental infection. One group of mice was left untreated. To evaluate the efficacy of mangiferin, from euthanised mice, 3- μ m-thick tissue sections of the intestine were stained with haematoxylin-eosin and periodic acid Schiff. Immunohistochemistry was also used by employing a monoclonal anti-*C. parvum* antibody. Oocysts were counted and results were expressed as mean oocysts number/intestine. Results obtained show that mangiferin at 100 mg/kg/die has a significant anticryptosporidial activity and that its activity is similar to that showed by the same dose (100 mg/kg/die) of paromomycin. However, both mangiferin and paromomycin were not able to completely inhibit intestinal colonization of *C. parvum* but only to reduce it. This reduction was calculated at over 80% for both mangiferin and paromomycin with respect to the untreated control. A significant activity was found also for mangiferin at 50 mg/kg/die only after the end of treatment.

S. Perrucci (✉) · G. Fichi · C. Buggiani
Dipartimento di Patologia Animale,
Profilassi ed Igiene degli Alimenti, University of Pisa,
Viale delle Piagge n° 2,
56124 Pisa, Italy
e-mail: perrucci@vet.unipi.it
Tel.: +39-050-2216949
Fax: +39-050-2216941

G. Rossi
Dipartimento di Scienze Veterinarie,
University of Camerino,
Camerino, Italy

G. Flamini
Dipartimento di Chimica Bioorganica e Biofarmacia,
University of Pisa,
Pisa, Italy

Introduction

Cryptosporidium parvum represents an important agent responsible for causing diarrhoea in both humans and domestic mammals (Casemore et al. 1997; Xiao et al. 2004). In humans, cryptosporidiosis, for immunocompetent hosts, is usually a self-limiting disease (Arrowood 1997). However, in children of developing countries and in immunocompromised hosts, this protozoan infection is attributed with a high mortality rate (Casemore et al. 1997; Fayer et al. 1997; O'Donoghue 1995). In immunocompromised hosts, extraintestinal spread of the infection can also be observed, and diarrhoea often persists and becomes life-threatening (O'Donoghue 1995). In children of developing countries, cryptosporidiosis is also considered an important cause of reduced growth rate and impaired cognitive function (Cacciò 2004; O'Donoghue 1995).

In animals, numerous case reports have described clinical cryptosporidiosis exhibiting primarily watery diarrhoea (O'Donoghue 1995). In livestock, cryptosporidiosis is both of clinical and economic concern (Casemore et al. 1997). Young animals appear to be more susceptible to clinical infection and a significant number of mortalities have been recorded in neonates of many species, especially ruminants (O'Donoghue 1995).

Although some symptomatic improvement has been reported in children and immunocompromised patients after treatment with spiramycin, paromomycin and azithromycin (Blagburn and Soave 1997; O'Donoghue 1995), there is a large consensus about the fact that no drug is consistently efficacious in therapy for cryptosporidiosis in humans (Blagburn and Soave 1997; O'Donoghue 1995; Theodos et al. 1998). Despite conflicting data, paromomycin has been frequently chosen as the first-line agent against cryptosporidial infection in patients with AIDS (Blagburn and Soave 1997). In animals, the most used drugs that show anticryptosporidial activity are represented by paromomycin, lasalocid, halofuginone and sulfaquinoxaline (Blagburn and Soave 1997) but, also in this case, no drug is consistently efficacious (Blagburn and Soave 1997; Theodos et al. 1998).



Coccidiosi nei piccoli ruminanti



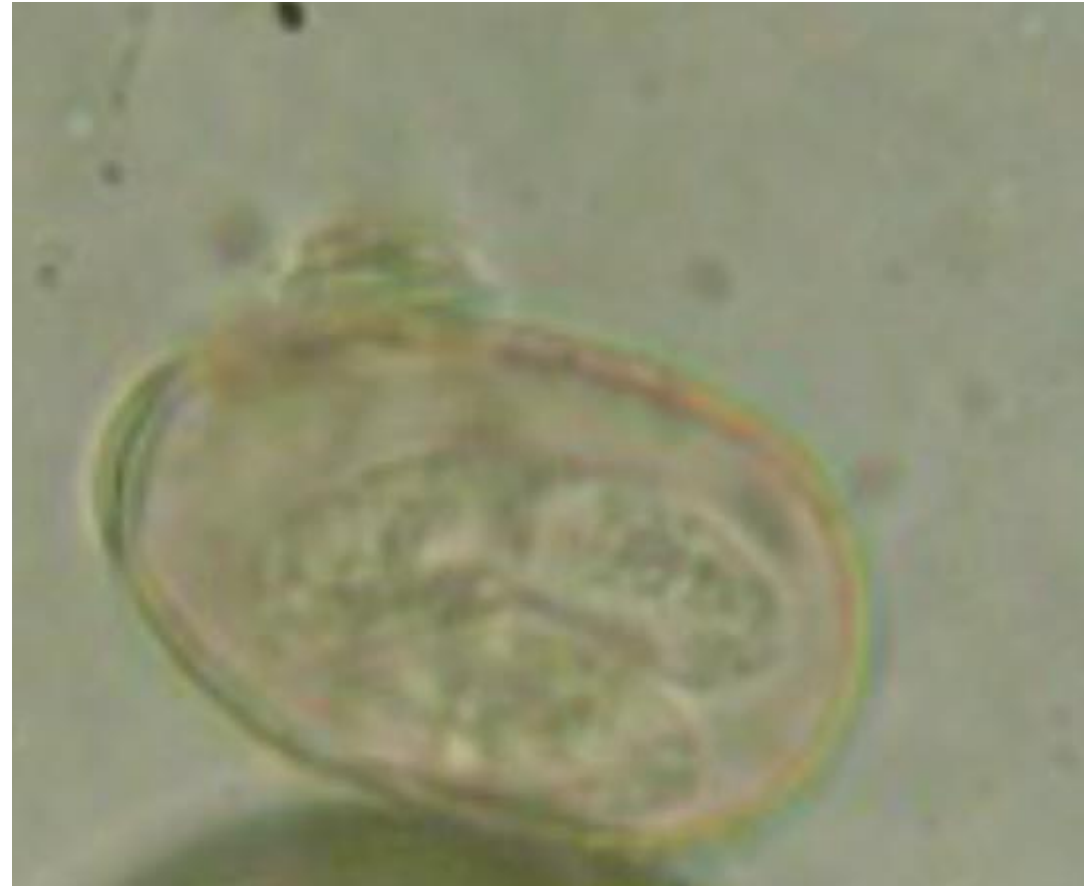
Animals	Eimeria species
Goats	<i>E. arloingi</i> , <i>E. ninakohlyakimovae</i> , <i>E. christenseni</i> , <i>E. caprovina</i> , <i>E. caprina</i> , <i>E. hirci</i> , <i>E. apsheronica</i> , <i>E. alijeve</i> , <i>E. jolchijevi</i> , <i>E. africensis</i> , <i>E. punctate</i> , <i>E. kocharli</i> , <i>E. capralis</i> , <i>E. masseyensis</i> , <i>E. charlestoni</i> , <i>E. minasensis</i>
Sheep	<i>E. ahsata</i> , <i>E. bakuensis</i> , <i>E. crandallis</i> , <i>E. faurei</i> , <i>E. granulosa</i> , <i>E. gonzalezi</i> , <i>E. gilruthi</i> , <i>E. intricata</i> , <i>E. marsica</i> , <i>E. ovinoideal</i> <i>is</i> , <i>E. pallida</i> , <i>E. parva</i> , <i>E. weybridgensis</i> , <i>E. punctate</i> , <i>E. dalli</i>

Parameters	Subclinical coccidiosis	Clinical coccidiosis
Poor feed conversion rate	+	+
Poor body weight gain	+	+
Weight loss	+	+
Intercurrent or concurrent diseases	+	+
Reduce productions (milk, wool, hair)	+	+
Losses due to mortality	-	+
Chemoprophylaxis	+	+
Treatments	+	+
Carcass deletion in slaughterhouse	-	+
Intestine deletion in slaughterhouse	-	+
Reduced fertility	+	+



Eimeria spp. oocisti matura

- La sporulazione avviene in 1-4 giorni in condizioni ambientali ottimali, ma può richiedere anche diverse settimane.
- Le oocisti sopravvivono fino a -8°C .
- Il fieno proveniente da pascoli contaminati può contenere un gran numero di oocisti infettanti anche dopo 8 mesi di conservazione.



Epidemiologia della coccidiosi dei ruminanti

La coccidiosi clinica è principalmente una malattia degli animali giovani, mentre è rara negli adulti.

Nelle pecore e capre le infezioni si verificano principalmente tra le 4-7 settimane di età.

Il colostro fornisce una protezione passiva durante le prime settimane di vita.



Epidemiologia della coccidiosi dei ruminanti

Prevalenza

Positivi 85-100% dei giovani animali

Le forme cliniche di solito si manifestano negli animali giovani nel periodo dello svezzamento (la sensibilità all'infezione massima si osserva intorno alle 4 settimane di età)

Le forme cliniche generalmente, ma non sempre, sono associate alla presenza di un elevato numero di oocisti nelle feci.

Generalmente negli allevamenti estensivi il livello di esposizione alle oocisti infettanti è più limitato, mentre nei sistemi di produzione al chiuso, gli animali giovani possono essere esposti ad ambienti fortemente contaminati

Epidemiologia della coccidiosi dei ruminanti

Prevalenza

Adulti: 20-80%

La coccidiosi clinica si può manifestare anche negli animali adulti in caso di:

1. infezioni massive
2. diminuita resistenza immunitaria a causa di situazioni di stress (sovraffollamento, cambiamenti di alimentazione, condizioni meteorologiche avverse)
3. alimentazione non corretta
4. gravi patologie concomitanti.



Epidemiologia della coccidiosi dei ruminanti

Prevalenza delle diverse specie



Country	Goat (Species/Prevalence)	References
Southern Portugal	<i>E. ninakohlyakimovae</i> (88%), <i>E. arloingi</i> (85%), <i>E. alijevei</i> (63%), <i>E. caprovina</i> (63%)	[62]
Czech Republic	<i>E. arloingi</i> (84%), <i>E. hirci</i> (63%), <i>E. ninakohlyakimovae</i> (56%), <i>E. christenseni</i> (55%)	[42]
Poland	<i>E. arloingi</i> (80%), <i>E. christenseni</i> (70%), <i>E. ninakohlyakimovae</i> (40%), <i>E. caprina</i> (20%)	[5]
Gran Canaria (Spain)	<i>E. ninakohlyakimovae</i> (30.0%), <i>E. arloingi</i> (28.6%), <i>E. alijevei</i> (20.5%), <i>E. caprina</i> (9.1%)	[60]
Nigeria	<i>E. jolchievi</i> (24%), <i>E. pallida</i> (22%), <i>E. arloingi</i> (18%), <i>E. apsheronica</i> (16%)	[71]
Zimbabwe	<i>E. alijevei</i> (99%), <i>ninakohlyakimovae</i> , (99%), <i>E. hirci</i> , (83.5%), <i>E. arloingi</i> , (80.6%)	[7]
Tanzania	<i>E. alijevei</i> (63%), <i>E. arloingi</i> (55%), <i>E. caprina</i> (26%), <i>E. ninakohlyakimovae</i> (26%)	[44]
South Africa	<i>E. arloingi</i> (97.47%), <i>E. hirci</i> (84.34%), <i>E. caprovina</i> (61.11%), <i>E. ninakohlyakimovae</i> (45.95%)	[23]
Southeastern Iran	<i>E. arloingi</i> (68.26 %), <i>E. christenseni</i> (50.9 %), <i>E. ninakohlyakimovae</i> (41.8 %), <i>E. caprina</i> (31.7 %)	[37]
Sri Lanka	<i>E. ninakohlyakimovae</i> (31%), <i>E. alijevei</i> (29%), <i>E. arloingi</i> (21%), <i>E. christenseni</i> (7%)	[16]
Malaysia	<i>E. arloingi</i> (71%), <i>E. ninakohlyakimovae</i> (67%) <i>E. christenseni</i> (63%) and <i>E. alijevei</i> (61%)	[31]
North of Jordan	<i>E. caprina</i> (13.5%), <i>E. ninakohlyakimovae</i> (12.5%), <i>E. arloingi</i> (11%), <i>E. apsheronica</i> (10%)	[1]
Northeastern China	<i>E. christenseni</i> (78.3%), <i>E. alijevei</i> (73.7%), <i>E. caprina</i> (62.3%), <i>E. arloingi</i> (44.6%)	[69]
Turkey (Van province)	<i>E. arloingi</i> (40.9%), <i>E. christensini</i> (34.3%), <i>E. alijevei</i> (32.6%), <i>E. pallida</i> (31.0%)	[13]
Turkey (Igdir province)	<i>E. arloingi</i> (47.43%), <i>E. christenseni</i> (45.14%), <i>E. ninakohlyakimovae</i> (36%), <i>E. alijevei</i> (26.85%)	[22]
Sheep (Species/Prevalence)		
Austria	<i>E. ovinoidalis</i> (28.3%), <i>E. crandallis</i> (27.3%), <i>E. weybridgensis</i> (27.3%), <i>E. ahsata</i> (19.1%)	[56]
Kenya	<i>E. bakuensis</i> (43.6%), <i>E. ovinoidalis</i> (23.6%), <i>E. ahsata</i> (15.2%), <i>E. intricata</i> (8.27%)	[52]
Tanzania	<i>E. crandallis</i> (96%), <i>E. parva</i> (92%), <i>E. ovinoidalis</i> (29%), <i>E. bakuensis</i> (29%)	[44]
Northwestern Iran	<i>E. intricate</i> (35%), <i>E. ovina</i> (18%), <i>E. faurei</i> (18%), <i>E. parva</i> (13%)	[75]
Western Iran	<i>E. ahsata</i> (81.46%), <i>E. parva</i> (67.46%), <i>E. pallida</i> (58.4%), <i>E. bakuensis</i> (56.26),	[27]
Northeastern China	<i>E. ahsata</i> (67.2%), <i>E. parva</i> (59.9%) and <i>E. bakuensis</i> (44.3%), <i>E. faurei</i> (17.1%)	[68]
Turkey (Kars province)	<i>E. ovinoidalis</i> (47.7%), <i>E. bakuensis</i> (46.6%), <i>E. parva</i> (37.1%), <i>E. granulosa</i> (27.7%)	[4]
Turkey (Antakya province)	<i>E. crandallis</i> (64.91%), <i>E. ovinoidalis</i> (55.24%), <i>E. bakuensis</i> (38.70%), <i>E. weybridgensis</i> (30.24%)	[35]
Saudi Arabia	<i>E. parva</i> (31.7%), <i>E. intricate</i> (26.8%), <i>E. arloingi</i> (22%), <i>E. ovina</i> (17.1%)	[66]

Epidemiologia della coccidiosi dei ruminanti

Fattori di rischio

- (i) Scarse condizioni igieniche
- (ii) Alimenti e acqua contaminati
- (iii) Fattori stressanti
- (iv) Condizioni di sovraffollamento
- (v) Pavimenti non lavabili
- (vi) Allevamento al chiuso
- (vii) Gregge di grandi dimensioni



Epidemiologia della coccidiosi dei ruminanti

Le principali fonti di infezione per agnelli e capretti sono:

- (i) Oocisti escrete dalle madri, in particolare nel periodo del parto**
- (ii) Oocisti che contaminano le aree di parto**
- (iii) Oocisti eliminate da altri agnelli/capretti**



Coccidiosi dei ruminanti

Controllo



Le misure di controllo dovrebbero mirare a:

1. Ridurre la contaminazione ambientale da oocisti
2. Evitare la contaminazione di acqua e alimenti
3. Fare in modo che gli animali diventino naturalmente resistenti alle forme cliniche
4. Evitare le condizioni di sovraffollamento e stress
5. Applicare misure igieniche efficaci
6. Evitare che gli animali giovani frequentino pascoli fortemente contaminati
7. Evitare di mantenere gruppi di età diversi nello stesso recinto
8. Proteggere gli animali da condizioni climatiche avverse
9. Alimentazione adeguata
10. Controllare le altre patologie

Coccidiosi dei ruminanti



Consumption of *Pistacia lentiscus* foliage alleviates coccidiosis in young goats

A. Markovics^a, I. Cohen^b, H. Muklada^b, T.A. Glasser^c, L. Dvash^b, E.D. Ungar^b, H. Azaizeh^d, S.Y. Landau^{b,*}

^a Department of Parasitology, Kimron Veterinary Institute, P.O. Box 12, Bet Dagan 50250, Israel

^b Department of Agronomy and Natural Resources, Institute of Plant Sciences, Agricultural Research Organization, the Volcani Center, P.O. Box 6, Bet Dagan 50250, Israel

^c The Ramat Hanadiv Nature Park, Zikhron Yaakov, Israel

^d Institute of Applied Research (Affiliated with University of Haifa), The Galilee Society, Shefa-Amr 20200, Israel

ARTICLE INFO

Article history:

Received 26 September 2011

Received in revised form

27 November 2011

Accepted 29 November 2011

Keywords:

Eimeria

Caprine

Tannin

Browse

Coccidiosis

Mediterranean

ABSTRACT

Coccidiosis near weaning is a major cause of diarrhea, ill-thrift, and impaired performance in small ruminants. A recent survey showed that in villages of the Samaria Hills, Israel, shepherds treat young, weaned goat kids afflicted with diarrhea by cutting and feeding them the foliage of *Pistacia lentiscus* L. (lentisk) or by tethering them close to lentisk bushes which they browse. The aim of the present study was to assess whether lentisk leaves do indeed have anti-coccidial value, and, if positive, to ascertain the role of tannins in this effect. We monitored for 24 (Experiment 1) and 30 (Experiment 2) days the effect of lentisk feeding on the development of naturally occurring coccidiosis in weaned kids artificially infected with parasitic nematodes. In Experiment 1, kids were infected with nematodes and fed lentisk foliage (PIS) or cereal hay (HAY). Coccidiosis developed at the early stage of the nematode infection, when dietary treatments were initiated. Kids in the PIS group had a lower ($P < 0.02$) concentration of oocysts per gram feces (opg). In Experiment 2, aimed at verifying if tannins are the active component in lentisk foliage, coccidiosis occurred at the peak of the nematode infection, before experimental diets were initiated. Dietary treatments were: cereal hay (HAY), or lentisk foliage consumed without (PIS) or with (PISPEG) a 20-g daily supplement of polyethylene glycol (PEG; MW 4000), a molecule that impairs tannin-bonding with proteins. Goats fed the PIS diet had lower fecal opg counts than counterparts of the HAY ($P < 0.001$) and PISPEG ($P < 0.002$) treatments. Fecal opg counts for the HAY and PISPEG treatments did not differ, suggesting that the anti-coccidial moiety in lentisk was indeed tannins. Our results strongly suggest that: (i) in agreement with the ethno-veterinary anecdotal evidence, exposure of young, weaned goat kids to lentisk foliage alleviates coccidiosis; and (ii) this positive effect is associated with tannins. As coccidiosis is a major affliction of kids, providing them with tannin-rich browse near weaning could be an environmentally friendly way of improving their welfare and health status, in particular under bio-organic farm management.

Coccidiosi dei ruminanti



ELSEVIER

Contents lists available at ScienceDirect

Small Ruminant Research

journal homepage: www.elsevier.com/locate/smallrumres



Effects of condensed tannin on natural coccidian infection in goat kids[☆]



C. Fraquelli, S.A. Zanzani, A.L. Gazzonis, R. Rizzi, M.T. Manfredi^{*}

Department of Veterinary Sciences and Public Health, Università degli Studi di Milano, Milan, Italy

ARTICLE INFO

Article history:
Available online 31 January 2015

Keywords:
Goat
Kids
Parasites
Coccidians
Eimeria
Condensed tannin

ABSTRACT

This study was carried out to test the efficacy of a particular type of Quebracho extract, rich in condensed tannins on coccidian infections and growth performances of kids naturally infected with *Eimeria* spp. Thirty-nine male kids aged 52–81 days were randomly allocated in 4 groups: A control group (CG, 9 kids) and three tannin-treated groups (TG, 10 kids each), that received a feed supplement containing Quebracho tannin (Silvafeed BYPRO Q). During the 45-day study period, kids received the treated feed, regularly once a day according to a varying schedule: For a week in a month (TG1), for a day every 5 days (TG2) and for 3 days every 15 days (TG3). Faecal samples were taken from each kid at 0, 5, 10, 15, 30, 35, 40, 45 days and individual oocyst counts per gram (OPG) were performed using the double FLOTAC[®] technique. In addition, animals were weighed at 15, 30 and 45 days of the trial. The results showed that the number of *Eimeria* oocysts in the treated groups decreased since the first 5 study days and was significantly different ($P < 0.0001$) from study 15-day period onwards. The percentage of oocyst reduction higher than 95% was found in 58% of the kids on study day 5. Comparison in OPG counts obtained from TG groups highlighted that in order to obtain a significant control of the *Eimeria* population, a high dose or a prolonged administration of Quebracho was required. In fact, OPG counts recorded in the TG1 kids receiving CT for 14 days resulted in the lowest oocyst count. Further, kids from TG2 recorded a lower least-square mean (LSM) value for OPG than the TG3 group showing that a single and more frequent administration of CT (TG2) seems to be more effective than treatments supplied repeatedly for 3 days (TG3). All kids consuming the CT weighed significantly heavier than the kids of the control group, and weight gain of kids from TG2 was higher than those of the other tannin-treated groups.

Coccidiosi dei ruminanti



The effect of sainfoin (*Onobrychis viciifolia*) and carob pods (*Ceratonia siliqua*) feeding regimes on the control of lamb coccidiosis

A. Saratsis¹ · N. Voutzourakis¹ · T. Theodosiou¹ · A. Stefanakis¹ · S. Sotiraki¹

Received: 9 February 2016 / Accepted: 17 February 2016 / Published online: 26 February 2016
© Springer-Verlag Berlin Heidelberg 2016

Abstract Recent research has suggested that plants containing condensed tannins may offer a promising alternative approach for the control of coccidiosis in lambs and goat kids. The present study aimed to examine the potential effect of condensed tannins in sainfoin (*Onobrychis viciifolia*) and carob pods (*Ceratonia siliqua*) incorporated in sheep rations against lamb coccidiosis. The above tannin-rich sources were studied in three independent feeding trials in which the animals (naturally infected by *Eimeria* spp. ewes and their lambs) were allocated (i) in the control group and received a tannin-free diet (lucerne hay), or (ii) in the treatment groups and received a tannin-rich diet based on sainfoin hay (in trials 1 and 2), or in carob pod meal and a combination of carob pod meal and sainfoin hay (in trial 3). In total, 95 newborn lambs (and their 73 ewes) were enrolled in all trials which started a month before lambing and ended 8–10 weeks after lambs were born (at weaning). The course of coccidial infection was monitored in lambs by faecal oocyst counts and consistencies which were recorded at weekly intervals. Moreover, lambs total weight gain was evaluated at the end of each trial. During all trials, 100 % of the animals got naturally infected by *Eimeria* species and the infection burden was higher in trials 2 and 3 compared to trial 1 but in all cases, severe signs of diarrhoea were not observed. Tannin-rich diets were well accepted by the animals not affecting their feed intake and body weight gain when compared to the controls. The results suggest that incorporation of both tannin-rich resources

(especially sainfoin) in sheep rations can reduce *Eimeria* oocyst excretion rates by the lambs, which can decrease subsequently the contamination of the farm environment with the parasite. However, the high variability noted on the results is not allowing us to draw any definite conclusions at least until the potential of those plants is further investigated.

Keywords *Eimeria* · Lamb · Coccidiosis · Condensed tannins · Sainfoin · Carob pods

Introduction

Coccidiosis of sheep is a widespread infection caused by the protozoan parasite, *Eimeria*, which develops in the small and the large intestine and affects young animals in particular, leading to considerable production and economic losses (Chartier and Paraud 2012; Taylor and Catchpole 1994). Infections with *Eimeria* are common in sheep worldwide, and clinical disease is frequently seen in young lambs that have been exposed to a high level of oocyst challenge. Subclinical infection can also have a negative impact on lamb growth rates (Gauly et al. 2004). Pathological changes are located in the intestine and include crypt hyperplasia, atrophy and necrosis of the villi leading to diarrhoea, decreased absorption of nutrients and as a consequence, reduced growth rates (Gregory et al. 1980; Berriatua et al. 1994).

Conventional *Eimeria* control strategies include the use of

Coccidiosi dei ruminanti



Sericea lespedeza as an aid in the control of *Emeria* spp. in lambs

J.M. Burke^{a,*}, J.E. Miller^b, T.H. Terrill^c, S.T. Orlik^b, M. Acharya^d, J.J. Garza^b, J.A. Mosjidis^e

^a United States Department of Agriculture, Agricultural Research Service, Booneville, AR 72927, USA

^b Louisiana State University, Baton Rouge, LA 70803, USA

^c Fort Valley State University, Fort Valley, GA 31030, USA

^d University of Arkansas, Fayetteville, AR 72901, USA

^e Auburn University, Auburn, AL 36849, USA

ARTICLE INFO

Article history:

Received 12 October 2012

Received in revised form

27 November 2012

Accepted 28 November 2012

Keywords:

Coccidia

Condensed tannins

Lambs

Sericea lespedeza

ABSTRACT

The objective was to examine the effects of feeding sericea lespedeza leaf meal (SL) on control of coccidiosis in lambs. In Exp. 1, naturally infected lambs ($n=76$) were weaned (102.7 ± 1.4 d of age) in May (spring) and randomly assigned in a 2×2 factorial design to receive 2% of BW/d of alfalfa pellets (control) or SL with or without amprolium added to drinking water ($n=38$ /level or 19/treatment). Fecal oocyst counts (FOC), egg counts (FEC), and fecal score (1 = solid pellets; 5 = slurry) were determined every 7 d between weaning and 21 d post-weaning. In Exp. 2, twin rearing ewes were randomly assigned to two groups, and their naturally infected lambs were fed a control creep supplement (16% CP; $n=40$) or SL pellets (14% CP; $n=32$) 30 d before weaning. Intake of SL was initially low (100 g/lamb daily) and increased to 454 g/lamb daily after weaning. Lambs were weaned at 103.6 ± 0.9 d of age and moved to semi-confinement. The FEC, FOC, packed cell volume (PCV), fecal score, and dag score (soiling around rear of lamb; 1 = no soiling; 5 = heavy soiling) were determined at d -14, 0 (weaning), 7, 14, and 21. In Exp. 3, lambs were randomly assigned to a control or SL diet ($n=12$ /diet) fed at 1.4 kg/d for 22 d and inoculated with 50,000 sporulated oocysts on d 8, 11, and 13. The FEC, FOC, and fecal score were determined every 2 to 3 d between d 1 and 29 (d 0 = first day of dietary treatment). Data on all experiments were analyzed using mixed models. The FOC and FEC data were log transformed. Chi squared analysis was used to determine differences in incidence of treatment (sulfadimethoxine) for coccidiosis in Exp. 1 and 2. In Exp. 1, FOC and FEC were similar between dietary groups, and FOC declined more rapidly in amprolium treated lambs following weaning ($P<0.001$). Fecal score was higher in the control compared with the SL fed lambs ($P=0.05$), suggesting more signs of coccidiosis in control lambs. In Exp. 2, FOC was similar initially but was reduced in SL fed lambs by weaning and remained lower thereafter ($P=0.004$). Dag ($P=0.01$) and fecal ($P=0.001$) scores were similar before weaning, but lower in SL fed lambs by weaning and remained lower thereafter. No SL lambs required treatment for coccidiosis, whereas 33% of control lambs required treatment ($P<0.001$). Fecal egg counts were similar before weaning but were reduced in SL compared with control fed lambs after weaning ($P<0.001$). In Exp. 3, FOC ($P<0.001$) and FEC ($P<0.001$) were reduced in SL compared with control fed lambs. Sericea lespedeza was effective in the prevention and control of coccidiosis as well as in reducing GIN infection. Use of SL could reduce lamb loss post-weaning, reduce the need to treat for coccidiosis, and create a significant economic benefit to livestock producers.

Coccidiosi dei ruminanti



Contents lists available at [ScienceDirect](https://www.sciencedirect.com)

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar



Research paper

Anticoccidial efficacy of naringenin and a grapefruit peel extract in growing lambs naturally-infected with *Eimeria* spp.



Agustín Pérez-Fonseca^a, Yazmin Alcala-Canto^a, Abdelfattah Z.M. Salem^{b,*}, Aldo B. Alberti-Navarro^c

^a Departamento de Parasitología, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, Mexico City, Mexico

^b Facultad de Medicina Veterinaria y Zootecnia, Universidad Autónoma del Estado de México, Toluca, Mexico

^c Departamento de Medicina y Zootecnia de Rumiantes, Facultad de Medicina Veterinaria y Zootecnia, Universidad Nacional Autónoma de México, Mexico City, Mexico

ARTICLE INFO

Article history:

Received 10 July 2016

Received in revised form 7 October 2016

Accepted 8 November 2016

Keywords:

Citrus

Coccidiosis *Eimeria* spp.

Grapefruit peel extract

Naringenin

ABSTRACT

The current study aimed to determine the anti-*Eimeria* efficacy of an extract of grapefruit peels (GF) and commercial naringenin (NAR) in naturally-infected lambs, as well as the influence of these flavonoids on the oxidative status during ovine coccidiosis. Pharmacokinetic profiles were also determined. Extracts were administered *per os* to *Eimeria* naturally infected growing lambs during 90 consecutive days. The commercial anticoccidial drug toltrazuril (TTZ) was included in this trial as a standard. Twenty-four lambs were divided into four groups: NAR, lambs given a daily dose of 5 mg of a commercial naringenin extract of 98% higher purity per kg body weight; GF, lambs that received a daily dose of 5 mg of ethanolic extract of grapefruit peels per kg body weight; TTZ, lambs treated with 20 mg of toltrazuril/kg body weight on days 0 and 15 of the experiment; and CTRL, untreated lambs that received daily dose of 30 ml of water. Daily doses of GF and NAR were dissolved in 30 ml of water and orally given to animals; whereas toltrazuril was administered as a single dose of an undiluted suspension to lambs of the TTZ group. The CTRL group received 30 ml of water; as well as the TTZ group for the period after the single dose administration. Fecal and serum samples were collected from all lambs. Anticoccidial efficacy was estimated by coprological techniques. Generation of nitric oxide levels and the antioxidant capacity of the experimental compounds were determined by the Griess and ABTS assays, respectively. The pharmacokinetic parameters of NAR and the GF extract were obtained. On day 30 post-ingestion, anticoccidial efficacy was 91.76% (NAR) and 89.65% (GF); whereas 99.63% of efficacy was achieved with TTZ 15 days after treatment. NAR, GF and TTZ significantly reduced oxidative stress in infected animals. The mean daily weight gain for each group was 122 g (NAR), 122 g (GF), 143 g (TTZ) and 98 g (CTRL). Following the oral administration of NAR and GF, values in plasma approached maximum concentrations within 2.1 to 2.5 h. In conclusion, the administration of NAR and the GF extract reduced *Eimeria* oocyst output, oxidative stress and promoted higher mean daily weight gains in infected lambs.

Coccidiosi dei ruminanti



Veterinary Parasitology 212 (2015) 5–17



Contents lists available at [ScienceDirect](#)

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar



Tannin containing legumes as a model for nutraceuticals against digestive parasites in livestock

H. Hoste^{a,b,*}, J.F.J. Torres-Acosta^c, C.A. Sandoval-Castro^c, I. Mueller-Harvey^d, S. Sotiraki^e, H. Louvandini^f, S.M. Thamsborg^g, T.H. Terrill^h

^a INRA, UMR 1225 IHAP, 23 Chemin des Capelles, Toulouse F-31076, France

^b Université de Toulouse, ENVT, 23 Chemin des Capelles, Toulouse F-31076, France

^c Campus de Ciencias Biológicas y Agropecuarias, FMVZ, Universidad Autónoma de Yucatán, Km 15.5 Carretera Mérida-Xmatkuil, Mérida, Yucatan, Mexico

^d University of Reading, School of Agriculture, Policy and Development, 1 Earley Gate, P.O. Box 236, Reading, RG6 6AT, United Kingdom

^e Veterinary Research Institute – Hellenic Agricultural Organization Demeter, 57001, Thessaloniki, Greece

^f Laboratory of Animal Nutrition, Centre for Nuclear Energy in Agriculture, University of São Paulo, Piracicaba, São Paulo, Brazil

^g Department of Veterinary Disease Biology, Faculty of Health and Medical Sciences, University of Copenhagen, Frederiksberg, Denmark

^h Fort Valley State University, 1005 State University Drive Fort Valley, GA 31030, USA

ARTICLE INFO

Article history:

Received 18 April 2015

Received in revised form 16 June 2015

Accepted 23 June 2015

Keywords:

Nutraceuticals

Condensed tannins

Polyphenols

Gastrointestinal nematodes

Antiparasitic effects

Small ruminants

ABSTRACT

Parasitic infections with gastrointestinal nematodes (GINs) still represent a worldwide major pathological threat associated with the outdoor production of various livestock species. Because of the widespread resistance to synthetic chemical anthelmintics, there is a strong impetus to explore novel approaches for a more integrated management of these infections. The use of nutraceuticals in the control of GINs is one of the alternatives which has been widely studied for 20 years.

The objectives of this review are: (i) to define and illustrate the concept of 'nutraceutical' in the context of veterinary parasitology based on data obtained on the most studied models to control GINs in small ruminants, the tannin-containing legumes (Fabaceae); (ii) to illustrate how the 'nutraceutical concept' could be expanded to other plants, other livestock production systems and other GI parasitic diseases, and (iii) to explain how this concept is opening up new research fields for better understanding the interactions between the host, the digestive parasites and the environment.

Fasciolosi

Fasciola hepatica è un distoma a localizzazione epatica, a livello dei dotti biliari e della cistifellea dei ruminanti.

Colpisce specialmente ovini, caprini, bovini e bufali, ma può infestare anche altri mammiferi, come equini, suini, cane, gatto e anche l'uomo.



In Europa, questa malattia nell'uomo è stata segnalata in Spagna, Portogallo, Francia e Italia. In Italia l'incidenza è di circa 10 casi/anno.

Nell'uomo il parassita rappresenta un problema sanitario importante in alcune aree del mondo, come il Nord Africa, il Medio-Oriente e, soprattutto, il Sud America dove si raggiungono prevalenze che sfiorano il 100%.

Fasciola hepatica

Epidemiologia

Nell'ultimo decennio, sono stati evidenziati aumenti di prevalenza di questa malattia in Europa, probabilmente connessi con i cambiamenti climatici:

nel Regno Unito la prevalenza della fasciolosi nei ruminanti raggiunge il 72%, in Svizzera è del 18%, in Germania è del 33% e in Austria del 67%.

Nel nord Europa il parassita non è molto diffuso, in quanto le temperature troppo rigide non facilitano lo sviluppo esogeno del parassita: la prevalenza nei paesi scandinavi è del 7%.

La prevalenza aumenta con l'età degli animali

Fasciola hepatica

Epidemiologia

in Italia sembra essere abbastanza diffusa:

Un recente studio condotto in Toscana ha evidenziato una prevalenza del 3-11% nei bovini e 11-19% negli ovini

Uno studio nelle zone appenniniche del Lazio, Molise e Abruzzo ha rivelato una prevalenza di 11% nei bovini e 5% negli ovi-caprini

Alcuni studi condotti nelle aree appenniniche meridionali hanno evidenziato una prevalenza di 1-11% negli ovini ma recentemente è stata osservata una maggiore incidenza di forme acute epidemiche con mortalità fino al 50%

Research Paper

Were natural forms of treatment for *Fasciola hepatica* available to the Etruscans?

Adrian P. Harrison¹✉, Jean M. Turfa²

1. Dept. of Animal & Veterinary Basic Sciences, Faculty of Life Science, University of Copenhagen, Grønnegaardsvej 7, 1870 Frederiksberg C, Denmark.
2. Mediterranean Section, University of Pennsylvania Museum of Archaeology and Anthropology, 3260 South Street, Philadelphia, PA 19104-6324, USA.

✉ Corresponding author: Dr A.P. Harrison (D.Phil. Cantab.), IBHV, Faculty of Life Sciences, Copenhagen University, Grønnegaardsvej 7, 1870 Frederiksberg C, Denmark. Tel: 0045 35332568, Fax: 0045 35332525, E-mail: adh@life.ku.dk

Received: 2010.07.12; Accepted: 2010.08.08; Published: 2010.08.09

Fasciolosi

Le perdite produttive sono interpretabili come:

- morte degli animali
- sequestro dei fegati al macello
- dimagrimento o mancato incremento ponderale (9%)
- riduzioni fino al 15% della produzione di latte



Distomatosi gastroenterica

- Il ciclo è molto simile a quello di *F. hepatica*.
- I molluschi ospiti intermedi, strettamente acquatici, appartengono ai generi *Bulinus* e *Planorbis*. In essi il ciclo si completa in 1-2 mesi secondo la specie in causa e le condizioni ambientali (T° ottimali sui 27°C).

Comunque

- Alcune specie possono utilizzare anche le limnee
- Le metacercarie resistono sul fieno

Epidemiologia simile a *F. hepatica*

Paramphistomidae



Fasciola hepatica



Fasciolosi e Paramfistomosi

Controllo



Fasciolosi e Paramfistomosi

Controllo



- Drenaggio dei pascoli e cura agronomica dei pascoli (riduzione habitat favorevoli a sviluppo *Limnea*);
- Evitare di far pascolare gli animali in aree fascioligene in momenti stagionali a rischio;
- Oppure se un'area presenta condizioni ambientali adatte per il ciclo di *F. hepatica* ma non è contaminata, portarci solo animali trattati da poco;
- Adeguata rotazione dei pascoli con recinzioni adeguate anche separando gli animali giovani dagli adulti;
- Risanamento adeguato del letame;
- Defecalizzazione ambientale.



Fasciolosi e Paramfistomosi Controllo



Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar



Research paper

Vaccination against *Fasciola hepatica* using cathepsin L3 and B3 proteases delivered alone or in combination

Agnieszka Wesołowska^a, Katarzyna Basałaj^a, Luke J Norbury^{a,b}, Alicja Sielicka^a, Halina Wędrychowicz^a, Anna Zawistowska-Deniziak^{a,*}

^a Włocławek Stajarski Institute of Parasitology, Polish Academy of Sciences, Towara 51/55, 00-818, Warsaw, Poland
^b School of Science, RMIT University, Bundoora, Victoria, 3083, Australia

ARTICLE INFO

Keywords:
Fasciola hepatica
Multivalent vaccine
Juvenile-specific antigens
Cathepsin L3
Cathepsin B3

ABSTRACT

No licensed vaccine is currently available for prevention of *Fasciola hepatica* infections. However, considering the alarming increase in drug resistance, there is an urgent need for a safe and fully effective vaccine against fasciolosis. Here, we tested if cathepsins L (FhCL3-1, FhCL3-2) and B (FhCB3) secreted by juvenile liver flukes are viable vaccine targets when delivered alone or in combination in a rat model. Since control over the early immune response is crucial for parasite's establishment in its host, it was hypothesised that targeting fluke juvenile stages may prove beneficial. Moreover, it was assumed that selected antigens will act in a cumulative manner to interfere with liver fluke migration and thereby will reduce *F. hepatica* infection. Recombinant FhCL3-1 and FhCL3-2 delivered alone reduced liver fluke burdens by 47 % and 63 %, respectively. A trivalent vaccine containing rFhCL3-1/CL3-2/CB3 did not increase the protective vaccine efficacy compared to the rFhCL3-2 vaccinated group (53 %), although, reductions in liver fluke wet weight (statistically significant) and liver damage score were most pronounced. Further, the highest IgG1 and IgG2a levels were seen in rFhCL3-2 vaccinated rats, the group for which the highest reduction in worm burden was demonstrated. Moreover, IgG1 and IgG2a levels in vaccinated rats were significantly elevated compared to those reported for control groups up to 4 week post-infection. While the mechanism of protection remains unknown, it appears that it depends on vaccine-induced antibodies directed against cathepsins. The obtained results imply that *F. hepatica* juvenile-specific cathepsins are promising vaccine candidates that induce responses that successfully target early migratory liver fluke stages. Now, the challenge is to evaluate these juvenile-specific cathepsins for use in livestock.

1. Introduction

Fasciola hepatica is a causative agent of fasciolosis, a disease that is not only an economic problem in livestock husbandry, but also poses a threat for human health (Cwiklinski et al., 2016). Since there is no approved vaccine against fasciolosis, liver fluke infection control relies almost exclusively on the use of anthelmintic drugs. However, high re-infection rates after drug treatment and the spread of triclabendazole resistance means this approach is no longer satisfactory (Kelley et al., 2016) and efforts to provide an effective vaccine against *F. hepatica* appear as a well-reasoned strategy. Among the many vaccine antigen candidates tested, *F. hepatica* cathepsins seem to be the most promising (Molina-Hernández et al., 2015). These proteins are involved in crucial parasite activities that facilitate parasitism, including tissue penetration, feeding, migration and immune evasion (Dalton et al., 2013). To date, vaccines containing cathepsins from adult liver fluke stages have

shown partial efficacy (Toet et al., 2014). Numerous DNA and protein (native and recombinant) cathepsin vaccine preparations have been tested in both laboratory and target species (Buffoni et al., 2012; Dalton et al., 1996; Golden et al., 2010; Kęsik-Brodacka et al., 2017; Piacenza et al., 1999; Villa-Mancera et al., 2014; Wesołowska et al., 2013; Wędrychowicz et al., 2007, 2003; Zafra et al., 2013). However, a safe and fully effective vaccine has not yet been developed.

Since control over early immune responses is fundamental for the parasites establishment in its host, targeting of juvenile stages may prove beneficial. Indeed, rat vaccination with an antigen fraction derived from Newly Excysted Juveniles (NEJs) induced strong protective immunity against *F. hepatica* infection (van Milligen et al., 2000). Moreover, an immunogenic protein referred to as procathesin L3 was identified and suggested to be an important target for early protection (Hammen et al., 2004; Reszka et al., 2005). Further, juvenile-specific cathepsin B and cathepsin L1 g (ortholog of cathepsin L3) vaccine



Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine



Vaccination of sheep with Quil-A® adjuvant expands the antibody repertoire to the *Fasciola* MF6p/FhHDM-1 antigen and administered together impair the growth and antigen release of flukes

Ricardo A. Orbegozo-Medina^a, Victoria Martínez-Sernández^a, Marta González-Warleta^b, José Antonio Castro-Hermida^b, Mercedes Mezo^b, Florencio M. Ubeira^{a,*}

^a Laboratorio de Parasitología, Facultad de Farmacia, Universidad de Santiago de Compostela, Santiago de Compostela, Spain
^b Laboratorio de Parasitología, Centro de Investigaciones Agrarias de Mabegondo, INAGAL, Abegondo, A Coruña, Spain

ARTICLE INFO

Article history:
Received 28 December 2017
Received in revised form 21 February 2018
Accepted 26 February 2018
Available online 8 March 2018

Keywords:
MF6p/FhHDM-1
Quil-A
Fasciola
Cathepsin L
Fasciolosis
Vaccine

ABSTRACT

Fasciolosis continues to be a major cause of economic losses in the livestock industry and a growing threat to humans. The limited spectrum of effective anthelmintics and the appearance of resistances urge the need for developing an effective vaccine. Most studies have been focused on the use of TH1-polarizing adjuvants and the use of recombinant *Fasciola* critical molecules and, despite the efforts, no reproducible protections have been achieved. The *F. hepatica* MF6p/FhHDM-1 protein is a heme-binding protein also reported to have immunomodulatory properties, constituting a promising target for vaccination and/or as target for the development of new flucicides. Thus, in this study, we investigated the effects of the TH1-polarizing adjuvant Quil A® on sheep immune response to MF6p/FhHDM-1, and the vaccine potential of both native and synthetic forms of this protein against ovine fasciolosis. Subcutaneous injection of Quil A® alone, i.e., without co-injecting any antigen, expands the antibody repertoire to MF6p/FhHDM-1 triggered by a subsequent primo-infection with metacercariae. This effect was not observed with aluminum hydroxide, the most frequently adjuvant used in commercial vaccines. On the other hand, vaccination with synthetic MF6p/FhHDM-1 in Quil A® prompted a 2–4-week delay in the antibody response induced in sheep by a challenge experimental infection. Moreover, fluke populations established showed stunted growth and low antigen release probably due to reduced metabolic activity. These observations suggest that primary circulating antibodies induced by the immunization had harmful effects on fluke development. Such effects could not be demonstrated to be associated to TH1 immune response linked events (production of IgG2 isotype antibodies and IFN-γ).

Dicroceliosi

Epidemiologia



- Parassitosi tipica dei pascoli asciutti;
- Uova molto resistenti (oltre 20 mesi), solo le alte T° associate a siccità le possono inattivare;
- Il parassita si sviluppa meglio nei molluschi adulti; infatti, un numero elevato di parassiti può determinare la morte del mollusco.
- Negli allevamenti ovini la prevalenza può raggiungere il 100%.

Dicroceliosi

Epidemiologia

- La prevalenza parassitologica e coproparassitologica ha carattere stagionale con aumento da settembre a dicembre e da febbraio a marzo rispettivamente;
- **Eventi immunodepressivi o stressanti, quali il trasporto, la transumanza, il sovraffollamento, determinano un aumento delle UPG da parte di pecore infestate.**

Dicroceliosi

Scala et al. *Parasites Vectors* (2019) 12:342
<https://doi.org/10.1186/s13071-019-3609-2>

Parasites & Vectors

RESEARCH

Open Access



Dicrocoeliosis in extensive sheep farms: a survey

Antonio Scala^{1,2†}, Claudia Tamponi^{1†}, Giorgia Dessì¹, Giampietro Sedda¹, Giuliana Sanna¹, Silvia Carta¹, Andrea Corda¹, Philippe Jacquet³, Antonio Varcasia^{1,2*}  and Ciriaco Ligios⁴

Abstract

Background: This study investigated the epidemiological and molecular aspects of dicrocoeliosis in extensive sheep farms.

Methods: From 2013 to 2014, copromicroscopical analyses in 190 dairy sheep farms and anatomo-pathological inspections in six slaughterhouses were carried in Sardinia, Italy. Rectal faecal samples were analyzed using the FLOTAC® method, and anatomo-pathological examinations were based on detecting thickened terminal bile ducts (TTBDs). In addition, genetic analyses were conducted on representative DNA samples of adult *Dicrocoelium* spp.

Results: Ninety-seven (51.1%) out of 190 sheep farms were coprologically positive for *Dicrocoelium* spp. In the liver, on the surface and cut surface, TTBDs were reported in 40.1% (309/770) and 15.3% (118/770) of the animals examined, respectively, with an overall prevalence of 25.5% (196/770). No intraspecific genetic variation was observed among the *Dicrocoelium dendriticum* isolates.

Conclusions: Our survey reveals the widespread presence of *D. dendriticum* in Sardinia, although seasonal, geographical and climatic conditions might be key factors in modulating the infection prevalence. Examining typical lesions due to *D. dendriticum* in the liver in abattoirs can be used as a marker for tracking chronic dicrocoeliosis infection.

Keywords: Sheep, Breeding, Trematoda, *Dicrocoelium dendriticum*, Epidemiology

Dicroceliosi

Gastrointestinal parasites, liver flukes and lungworms in domestic ruminants from central Italy



E. SECCHIONI, M. SGORBINI, S. PERRUCCI*

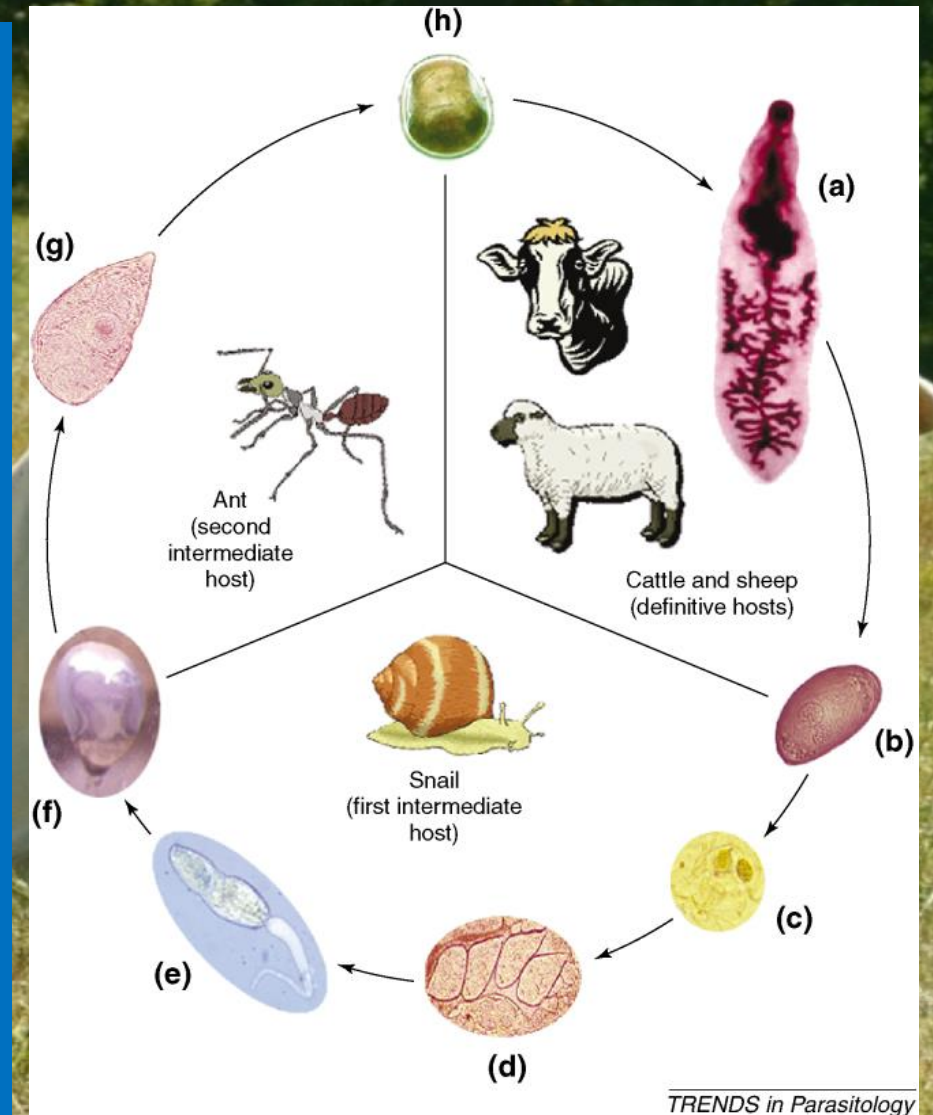
. Large Animal Review 2016; 22: 195-201 199

Table 3 - Prevalence of isolated gastrointestinal, lung and liver parasitic species observed by faecal analysis in cattle, sheep and goats from flat areas subject to water stagnation during rainy periods (A1) and from hilly and drier areas (A2).

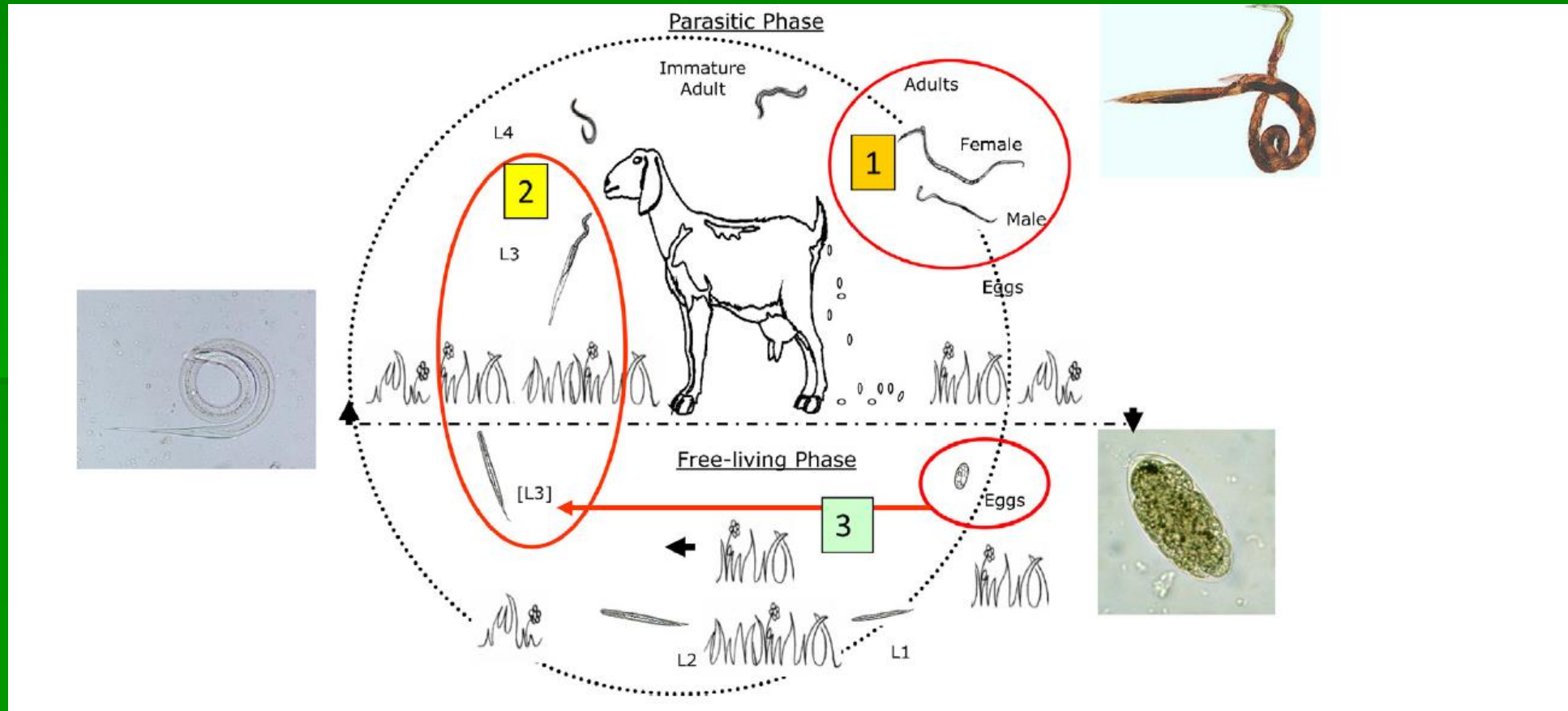
Parasites	Overall	A1	A2	Cattle	Sheep	Goats
Gastronitestinal strongyles	71.3% (127/178)	63.4% (71/111)	83.6% (56/67)	52.6% (40/76)	85.2% (52/61)	85.4% (35/41)
<i>Strongyloides papillosus</i>	10.7% (19/178)	5.4% (6/111)	19.4% (13/67)	5.3% (4/76)	18.1% (11/61)	9.8% (4/41)
<i>Trichuris</i> spp.	12.4% (22/178)	8.1% (9/111)	19.4% (13/67)	0% (0/76)	19.7% (12/61)	24.4% (10/41)
<i>Capillaria</i> spp.	2.2% (4/178)	0.9% (1/111)	4.5% (3/67)	5.3% (4/76)	0% (0/61)	0% (0/41)
Lungworms	10.1% (18/178)	3.6% (4/111)	20.9% (14/67)	0% (0/76)	18% (11/61)	17.1% (7/41)
<i>Fasciola hepatica</i>	0% (0/178)	0% (0/111)	0% (0/67)	0% (0/76)	0% (0/61)	0% (0/41)
<i>Dicrocoelium dendriticum</i>	14% (25/178)	9.1% (10/111)	22.4% (15/67)	0% (0/76)	32.8% (20/61)	12.2% (5/41)
<i>Eimeria</i> spp.	56.7% (101/178)	43.2% (48/111)	79.1% (53/67)	26.3% (20/76)	75.4% (46/61)	85.4% (35/41)
<i>Buxtonella sulcata</i>	8.9% (16/178)	1.8% (2/111)	20.9% (14/67)	21% (16/76)	0% (0/61)	0% (0/41)
<i>Moniezia benedeni</i>	3.4% (6/178)	2.7% (3/111)	4.5% (3/67)	0% (0/76)	6.5% (4/61)	4.9% (2/41)

Dicrocoeliosi Controllo

- Trattamenti strategici in pieno inverno
- **Difficoltà di un controllo ambientale ma si può intervenire con:**
- Rotazioni e rinnovo cotica erbosa (solo parzialmente efficace)
- Evitare il pascolo nelle prime ore del mattino ed al crepuscolo
- Controllo biologico: oche e tacchini che si nutrono degli ospiti intermedi.



Controllo Strongilosi gastrointestinale (e *Dictyocaulus filaria*)



Controllo Integrato.....

Finalità

- abbassare la carica parassitaria degli animali a valori tali da permettere una stimolazione immunitaria senza avere ripercussioni cliniche evidenti, limitando il più possibile il danno produttivo e l'uso di farmaci di sintesi
- Per ottenere questo risultato esiste la necessità di utilizzare un approccio che prevede l'uso contemporaneo di diverse tipologie di intervento.

Controllo integrato.....

“Le diverse tecniche utilizzate nel controllo integrato sono finalizzate sia a ridurre la possibilità di contatto tra parassita e ospite, sia ad aumentare la capacità di quest’ultimo di rispondere efficacemente ad una infezione parassitaria”

Strongilosi gastrointestinale

Controllo Integrato

- Gestione pascolo (varie tipologie di rotazione dei pascoli; pascolo in avanti dei giovani; scelta di rientro e di uscita al pascolo nel corso della giornata)
- numero di animali per unità di superficie (carico istantaneo)
- alimentazione
- scelta delle razze resistenti
- Trattamenti alternativi
- Controllo biologico
- vaccini

Controllo integrato.....

Presupposti fondamentali per il successo di questo tipo di controllo sono il rispetto del benessere degli animali, l'uso di norme igieniche (lavaggio e disinfezione delle strutture e attrezzature, uso razionale della lettiera, defecalizzazione ambientale...) ed il contemporaneo controllo delle patologie concomitanti.

Controllo integrato.....

Gestione del pascolo

- Cura agronomica
- Ritmo di pascolamento giornaliero
- Densità di animali per unità di superficie, anche come carico istantaneo
- Rotazione

Controllo integrato.....

Rotazione

- **Metodi preventivi:** impedire agli animali di infestarsi (soprattutto i giovani) utilizzando pascoli non contaminati o poco contaminati (a riposo da un anno, coltivati in precedenza o utilizzati da animali di specie diversa), pascolo in avanti per i giovani
- **Metodi di diluizione:** diluire la carica ambientale in modo che gli animali non si infestino con cariche elevate (uso di animali di età o specie diversa nello stesso pascolo)
- **Metodi evasivi:** evitare le infezioni massive e cliniche per uso di aree molto contaminate spostando gli animali in un'altra area -parcellizzazione del pascolo con permanenza sulla stessa parcella per un tempo non superiore al tempo medio di sviluppo delle larve infestanti dalle prime feci (tempo che dipende dalla stagione e dalle condizioni ambientali-3 settimane a 10-13°C, massimo 2 settimane a 15-20°C, meno di 10 giorni a 22°C, meno di 7 giorni a 24-25°C, circa un mese a 9-12°C), con riutilizzo della stessa parcella dopo tre mesi- un anno ma che può essere utilizzata da animali di specie diversa o coltivata.

Controllo integrato.....

Rotazione

- Un metodo che si è rivelato utile in Francia è il pascolo misto con i bovini:
- Area di pascolo divisa in due settori principali, una per le pecore dallo svezzamento degli agnelli al parto e l'altra per i bovini e gli agnelli. Dopo il parto si fa il cambio dei settori. L'area destinata agli agnelli è poi divisa in parcelle su cui gli agnelli sostano per 1-2 settimane e non vi tornano prima di 60 giorni, intervallo in cui le parcelle sono utilizzate solo dai bovini.
- Altri sistemi utilizzati per i ruminanti prevedono di alternare capre e pecore con i bovini sui pascoli ad intervalli di 2-6 mesi oppure di un anno alternando ovini-bovini-coltivazione

Possibilità di alternanza sui pascoli dei ruminanti con altre specie: cavalli e maiali.

Controllo integrato.....

Alimentazione

1. Gli animali spesso si infettano/infestano tramite l'alimento
2. I parassiti determinano alterazioni metaboliche
3. Alimentazione ha effetti su resilienza e resistenza alle parassitosi
 - Infatti, molte ricerche dimostrano che negli animali da reddito una migliore nutrizione riduce le perdite produttive e la mortalità dovute ai parassiti.
 - L'alimentazione degli animali può rappresentare una valida alternativa alla chemioprolifassi per il controllo delle parassitosi degli animali.

Controllo integrato.....

Alimentazione

- **L'alimentazione può influire sullo sviluppo e le conseguenze del parassitismo in tre diversi modi:**
 1. **Aumenta la capacità dell'ospite di superare gli effetti negativi causati dai parassiti (resilienza);**
 2. **Migliora la capacità dell'ospite di limitare lo stabilirsi, il grado di crescita, la fecondità e/o la persistenza di una popolazione parassitaria (resistenza);**
 3. **Contenendo composti ad effetto antielmintico diretto.**

Controllo integrato.....

Alimentazione

- Si deve considerare che l'alimentazione può presentare contemporaneamente tutti e tre questi tipi di effetto.
- **Proteine, oligoelementi e sali minerali:** potenziano la risposta immunitaria (Se, Zn, Mb) oppure azione diretta sui parassiti (P)

Controllo integrato.....

Alimentazione

- **Integrazioni alimentari proteiche “strategiche”**, in particolare alle categorie più sensibili dell'allevamento come i giovani e le femmine adulte nel periodo del parto (gravidanza e lattazione), può apportare benefici a lungo termine.

Ad esempio, negli animali con integrazione alimentare: peri-parturient rise ridotto o assente e nei giovani minor intensità delle parassitosi, minori danni

- Relazione **alimentazione-razze resistenti** per un miglior utilizzo dell'alimento disponibile

Controllo integrato..... Alimentazione

Un importante argomento è rappresentato dall'interazione tra i minerali e gli oligoelementi e la resistenza degli animali da reddito ai parassiti.

- Un aumento del contenuto di **fosforo** nella dieta di pecore sperimentalmente infettate con *Trichostrongylus vitrinus*, un nematode a localizzazione intestinale, è risultata capace di ridurre del 50% le UPG (Uova Per Grammo di feci) e dell'89% il numero di esemplari di questa specie parassitaria. Questi risultati fanno ipotizzare un effetto antielmintico diretto di questo minerale o, più probabilmente, essi possono riflettere alcuni cambiamenti metabolici derivanti da un basso assorbimento di fosforo negli animali parassitati.

Controllo integrato.....

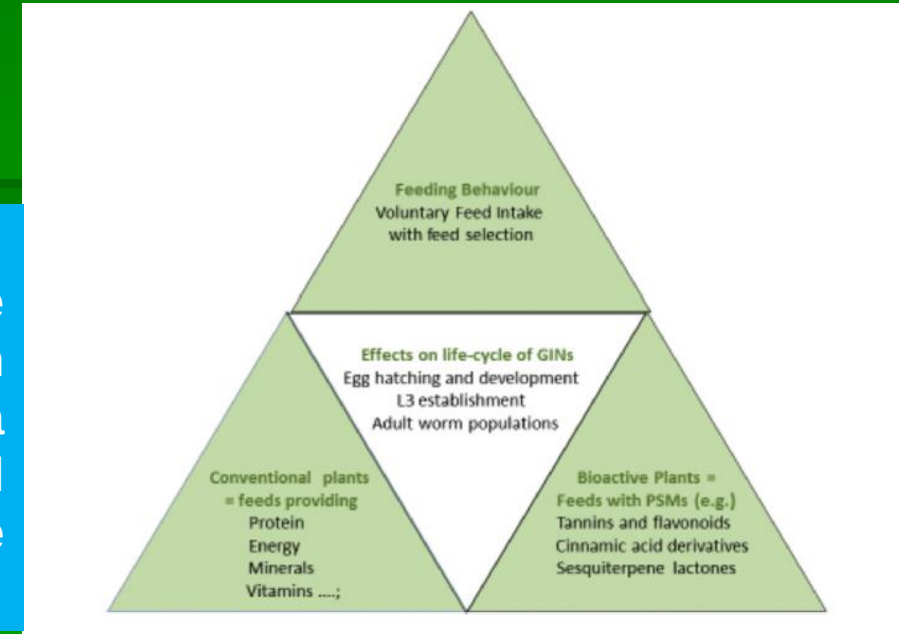
Alimentazione

- Il **molibdeno** è un oligoelemento che si è dimostrato capace di influenzare la popolazione di SGE (Strongili Gastro-Enterici) nelle pecore, sia con un **effetto diretto sul metabolismo dei nematodi**, sia per la sua possibile **capacità di aumentare la risposta infiammatoria nella mucosa intestinale**.
- Anche il **cobalto** ed il **selenio** sembrano possedere una certa attività antielmintica.

Controllo integrato...Nutraceutici e Fitoterapici

Nutraceutici

Un nutraceutico è "qualsiasi sostanza che può essere considerata un alimento o parte di un alimento che fornisce benefici per la salute, compresa la prevenzione e il trattamento delle malattie» (Andlauer e Fürst, 2002).



I nutraceutici sono coltivazioni (piante) contenenti metaboliti secondari (nutricine) utilizzate per i loro benefici effetti sulla salute degli animali piuttosto che a scopi nutritivi.

Essi possono essere utilizzati dagli animali (ruminanti) sia al pascolo che previa conservazione con il fine principale di ridurre le infezioni parassitarie

Controllo

integrato.....Nutraceutici e fitoterapici

Nutraceutici

- Tra i composti di origine vegetale che negli ultimi anni sino stati studiati per le loro proprietà antielmintiche, possono essere inserite le piante contenenti **tannini condensati**.
- I tannini sono un gruppo eterogeneo di composti fenolici, naturalmente presenti in diverse varietà di essenze foraggere consumate dagli animali, soprattutto nelle leguminose (sulla, ginestrino..), così come nel legno di alcune piante. Essi sono considerati delle difese chimiche contro i patogeni, quali i funghi, o gli erbivori stessi date le loro proprietà tossiche che possiedono ed il loro sapore sgradevole.
- I due gruppi più importanti in cui questi composti possono essere distinti sono quello dei **tannini idrolizzabili e quello dei tannini condensati**.

Controllo integrato.....

- **È stato osservato che pecore parassitate e alimentate con tannini condensati mostrano una riduzione nell'eliminazione di uova degli strongili gastrointestinali con le feci (UPG)**
- La riduzione delle UPG è osservabile già dopo due giorni dalla somministrazione, paragonabile al periodo d'azione dei tradizionali farmaci antielmintici.
-
- Però, se per i farmaci si può ottenere una riduzione del 95-100%, con l'utilizzo dei tannini la diminuzione è più limitata e contenuta, pari a circa il 60% ma che può arrivare al 90%
- **I tannini condensati sembrano avere effetti anche sulle larve infettanti che penetrano nell'ospite**

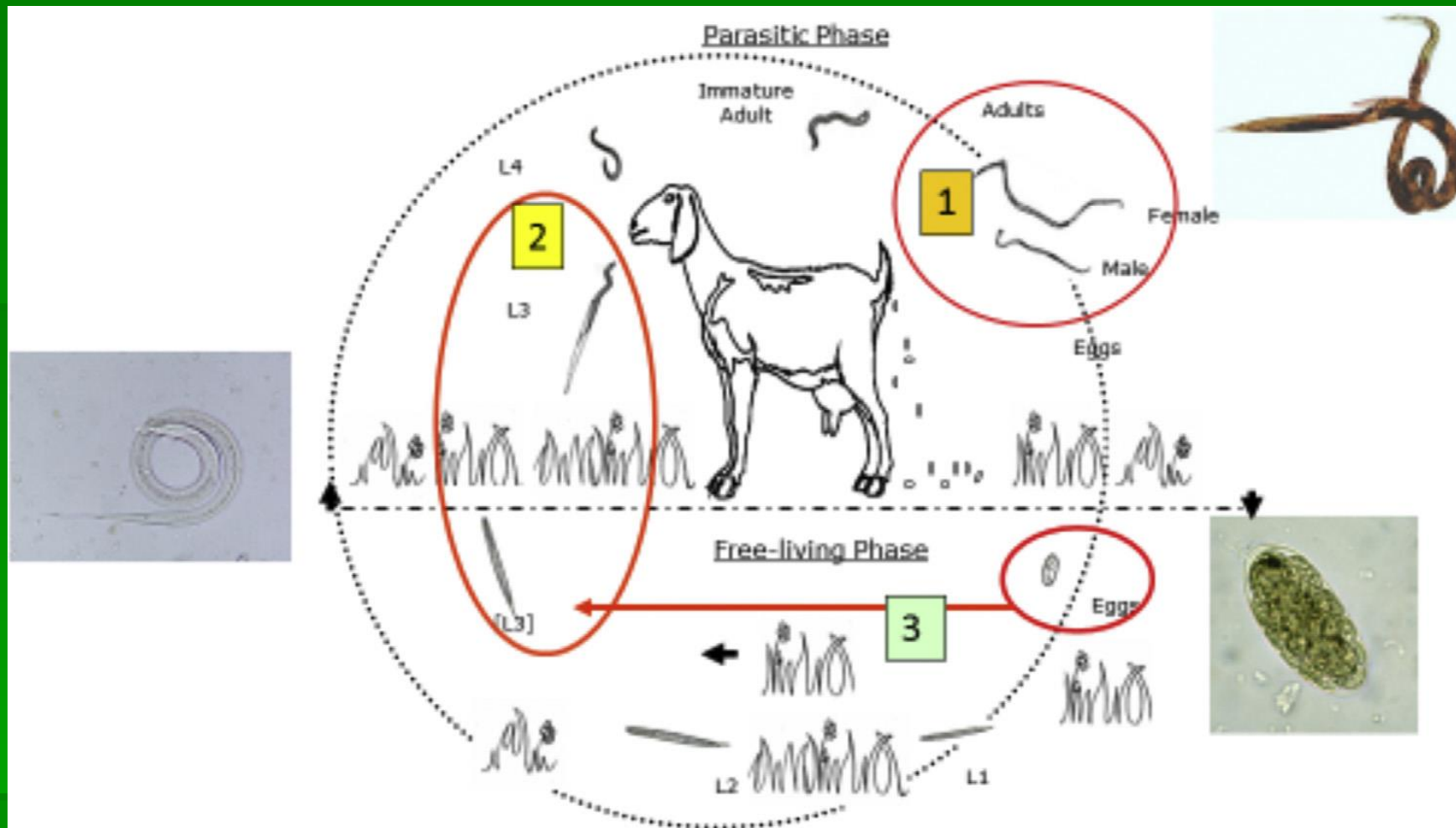
Controllo integrato.....

- L'attività antielmintica diretta dei tannini condensati sarebbe dovuta:

1. alla loro capacità di legare le sostanze proteiche, da cui deriva una limitazione per la nutrizione dei parassiti, sia larve che adulti, portandoli alla morte.

2. alla formazione di complessi molecolari capaci di determinandone la lisi della mucosa intestinale e della cuticola dei parassiti.

La riduzione delle Uova Per Grammo (UPG) eliminate con le feci degli animali sono osservabili dopo un periodo di 2-7 giorni di somministrazione dei tannini condensati.



Three key stages of the GIN life cycle have been identified as possible targets when tanniniferous plants are consumed by infected small ruminants: (1) a reduced excretion of nematode eggs by the adult worms (maximum values up to 80% reduction) (Shaik et al., 2006); (2) a reduced establishment of the infective third-stage larvae in the host (up to 70%) (Brunet et al., 2008); and (3) a reduced development of eggs to third-stage larvae (maximum values of reduction up to 90%) (Niezen et al., 2002).



Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar



Tannin containing legumes as a model for nutraceuticals against digestive parasites in livestock

H. Hoste^{a,b,*}, J.F.J. Torres-Acosta^c, C.A. Sandoval-Castro^c, I. Mueller-Harvey^d, S. Sotiraki^e,
H. Louvandini^f, S.M. Thamsborg^g, T.H. Terrill^h

^a INRA, UMR 1225 IHAP, 23 Chemin des Capelles, Toulouse F-31076, France

^b Université de Toulouse, ENVT, 23 Chemin des Capelles, Toulouse F-31076, France

^c Campus de Ciencias Biológicas y Agropecuarias, FMVZ, Universidad Autónoma de Yucatán, Km 15.5 Carretera Mérida-Xmatkuil, Mérida, Yucatan, Mexico

^d University of Reading, School of Agriculture, Policy and Development, 1 Earley Gate, P.O. Box 236, Reading, RG6 6AT, United Kingdom

^e Veterinary Research Institute – Hellenic Agricultural Organization Demeter, 57001, Thessaloniki, Greece

^f Laboratory of Animal Nutrition, Centre for Nuclear Energy in Agriculture, University of São Paulo, Piracicaba, São Paulo, Brazil

^g Department of Veterinary Disease Biology, Faculty of Health and Medical Sciences, University of Copenhagen, Frederiksberg, Denmark

^h Fort Valley State University, 1005 State University Drive Fort Valley, GA 31030, USA

ARTICLE INFO

Article history:

Received 18 April 2015

Received in revised form 16 June 2015

Accepted 23 June 2015

Keywords:

Nutraceuticals

Condensed tannins

Polyphenols

Gastrointestinal nematodes

Antiparasitic effects

Small ruminants

ABSTRACT

Parasitic infections with gastrointestinal nematodes (GINs) still represent a worldwide major pathological threat associated with the outdoor production of various livestock species. Because of the widespread resistance to synthetic chemical anthelmintics, there is a strong impetus to explore novel approaches for a more integrated management of these infections. The use of nutraceuticals in the control of GINs is one of the alternatives which has been widely studied for 20 years.

The objectives of this review are: (i) to define and illustrate the concept of ‘nutraceutical’ in the context of veterinary parasitology based on data obtained on the most studied models to control GINs in small ruminants, the tannin-containing legumes (Fabaceae); (ii) to illustrate how the ‘nutraceutical concept’ could be expanded to other plants, other livestock production systems and other GI parasitic diseases, and (iii) to explain how this concept is opening up new research fields for better understanding the interactions between the host, the digestive parasites and the environment.

Relative abilities of young sheep and goats to self-medicate with tannin-rich sainfoin when infected with gastrointestinal nematodes

E. Gaudin^{1,2,3}, M. Costes-Thiré¹, J. J. Villalba⁴, H. Hoste³, V. Gerfault² and C. Ginane^{1†}

¹Université Clermont Auvergne, INRA, VetAgroSup, UMR Herbivores, F-63122 Saint-Genès-Champagnelle, France; ²MG2MIX, La basse haye, F-35220 Châteaubourg, France; ³IHAP, Université de Toulouse, INRA, ENVY, 21 chemin des capelles, F-31000 Toulouse, France; ⁴Quinney College of Natural Resources, Utah State University, 5230 Old Main Hill, Logan, UT 84322, USA

(Received 24 April 2018; Accepted 4 October 2018; First published online 13 November 2018)

Plant secondary metabolites (PSM) are one of the promising options to control gastrointestinal nematodes in sheep and goats. The objective of this study was to assess the abilities of sheep and goats to self-medicate with tannin-rich sainfoin (*Onobrychis viciifolia*) (SF) when infected with gastrointestinal nematodes, using a cafeteria and an operant conditioning trial. Hypotheses were that parasitized (P) lambs and goat kids would show greater intake and preference for SF than their non-parasitized (NP) counterparts, that kids would eat more SF than lambs (due to their lower resistance against parasites and their greater ability to consume PSM), and that SF intake would increase over time for P animals. We used 20 female kids and 20 ewe lambs aged 3 months. Half of the animals per species ($n = 10$) were experimentally infected with 170 L₃ larvae of *Haemonchus contortus*/kg of BW (P). The other half were free from parasites throughout the study (NP). Five weeks after infection, animals were exposed to a 24-day cafeteria trial (three 8-day periods) offering a free choice between two legume pellets: SF (3.8% condensed tannins) and alfalfa (ALF, *Medicago sativa*; no tannin). Subsequently, animals were involved in an operant conditioning trial of two 4-day long sessions, to assess in short-term tests their motivation to walk for a SF reward when offered in choice with freely available ALF. In the cafeteria trial, SF preference was greater in kids than in lambs, particularly in the first two periods. We did not observe a greater preference for SF in P animals, which was even greater in NP animals for periods 1 and 2. Sainfoin intake increased through periods for P animals, which led to similar SF preferences for all groups during period 3. In the operant-conditioning trial, motivation to get the SF reward was similar between P and NP animals. These results support the hypotheses that goats are more willing to consume tanniferous feeds than sheep, and that P animals increased SF intake through time. However, the emergence of a curative self-meditative behaviour was not supported, as P individuals did not show greater SF intake, preference, nor a greater motivation to get SF than NP animals, regardless of animal species. These findings are discussed with previous results and some explanations are presented.

REVIEW ARTICLE

OPEN ACCESS

Ruminant self-medication against gastrointestinal nematodes: evidence, mechanism, and origins[☆]

Juan J. Villalba^{1,*}, James Miller², Eugene D. Ungar³, Serge Y. Landau³, and John Glendinning⁴

¹ Department of Wildland Resources, Utah State University, 5230 Old Main Hill, Logan, Utah 84322-5230, USA

² Department of Pathobiological Sciences, School of Veterinary Medicine, Louisiana State University, 70803 Baton Rouge, USA

³ Department of Natural Resources, Institute of Plant Sciences, Agricultural Research Organization, the Volcani Center, Bet Dagan 50250, Israel

⁴ Department of Biology, Barnard College, Columbia University, 3009 Broadway, New York, NY, USA

Received 10 January 2014, Accepted 17 June 2014, Published online 30 June 2014

Abstract – Gastrointestinal helminths challenge ruminants in ways that reduce their fitness. In turn, ruminants have evolved physiological and behavioral adaptations that counteract this challenge. Ruminants display anorexia and avoidance behaviors, which tend to reduce the incidence of parasitism. In addition, ruminants appear to learn to self-medicate against gastrointestinal parasites by increasing consumption of plant secondary compounds with antiparasitic actions. This selective feeding improves health and fitness. Here, we review the evidence for self-medication in ruminants, propose a hypothesis to explain self-meditative behaviors (based on post-ingestive consequences), and discuss mechanisms (e.g., enhanced neophilia, social transmission) that may underlie the ontogeny and spread of self-meditative behaviors in social groups. A better understanding of the mechanisms that underlie and trigger self-medication in parasitized animals will help scientists devise innovative and more sustainable management strategies for improving ruminant health and well-being.

Controllo integrato.....

- Anche una specie di cicoria (*Cichorium intybus*) ha effetti antielmintici nei confronti dei nematodi dei ruminanti (strongili) che se ne alimentino che includono anche le specie abomasali e polmonari.
- I principi attivi della cicoria sembrano essere i lattoni sesquiterpenici che hanno dimostrato proprietà antielmintiche *in vitro*.

Il problema principale dei nutraceutici è legato al fatto che i metaboliti secondari di una pianta variano sia nella composizione che nella quantità in base a numerosi fattori (terreno, clima, tipo di conservazione..etc) che possono rendere altamente variabili i loro effetti.

REVIEW



Cite this: *Nat. Prod. Rep.*, 2017, 34, 627

Medicinal plant extracts and plant-derived polyphenols with anthelmintic activity against intestinal nematodes†

V. Spiegler,^a E. Liebau ^b and A. Hensel ^{*a}

Covering: 2001 up to the end of 2016

Polyphenols comprise a structurally diverse class of natural products. As the development of new anthelmintic drugs against soil-transmitted helminthiasis is an urgent need and polyphenols are widely used in the treatment of nematode infections in traditional medicine and modern science, we summarize the state of knowledge in the period of mainly 2001 up to the end of 2016 on plant extracts with known polyphenolic composition and of defined polyphenols, mainly from the classes of condensed and hydrolysable tannins, flavonoids, and phenylpropanoids. The diverse biological activity against different helminths and the underlying mechanisms are reviewed.

Received 28th December 2016

DOI: 10.1039/c6np00126b

rsc.li/npr

1. Introduction
2. Anthelmintic activity of plant extracts and fractions enriched in polyphenols
 - 2.1. *In vitro* investigations
 - 2.2. *In vivo* investigations
3. Studies using phytochemically characterized fractions or pure compounds
 - 3.1. Condensed tannins
 - 3.1.1. The building blocks of CTs: flavan-3-ols
 - 3.1.2. Oligomeric proanthocyanidins
 - 3.2. Hydrolysable tannins
 - 3.3. Flavonoids
 - 3.4. Phenylpropanoids and related compounds

are roundworms (*Ascaris lumbricoides*), whipworms (*Trichuris trichiura*) and hookworms (*Ancylostoma duodenale* and *Necator americanus*)^{2–4} which commonly occur as coinfections.⁵ An infection usually starts with the ingestion of embryonated eggs by the host, or, in case of hookworms, with the infective third-stage larvae penetrating into the host's skin. Larval stages will then migrate through several tissues until they finally develop into adults when reaching the small intestine.⁶ Although usually not lethal, mainly adult worms that reside in the intestine cause severe symptoms, especially in children, such as anaemia and retardation in growth and cognitive development.^{1,3,7} In livestock, infestations with gastro-intestinal (GI) nematodes similarly lead to morbidity rather than mortality of the respective animals along

Controllo integrato.....

Fitoterapici (formulazioni a base di estratti o principi attivi ottenuti da piante e utilizzati a scopi terapeutici)

- Spesso elevata tossicità (dose efficace vicina alla dose massima tollerata)
- Spesso poco efficaci
- Spesso attività non dimostrata scientificamente

Tra i principi vegetali di maggiore interesse ci sono diversi gruppi di composti: **tannini, flavonoidi, saponine, terpeni e le proteinasi della cisteina**. Ad esempio, questi ultimi determinano dei danni sulla cuticola dei nematodi causandone la morte. Essi sono contenuti in grosse quantità nella papaya, nell'ananas e nei fichi.

Controllo integrato.....

Resistenza genetica

- Scelta delle razza: **utilizzo di razze autoctone**
- **Selezione dei capi resistenti all'interno dell'allevamento principalmente con la stima delle FECRT** (spesso però correlazione negativa tra resistenza e produttività)
- Valutazione delle razze resistenti
- Studi mirati ad identificare i geni responsabili di resistenza

Controllo integrato.....

Controllo biologico

Il controllo biologico è un metodo in cui l'uomo utilizza antagonisti viventi e naturali allo scopo di mantenere le popolazioni parassitarie ad un livello non pericoloso/dannoso per gli animali e le produzioni animali.

Questo metodo si applica principalmente per il controllo degli stadi liberi nell'ambiente, negli ospiti intermedi e nei vettori, ma anche negli animali nel caso degli ectoparassiti.

Nel caso degli endoparassiti, indirettamente si ottiene anche una riduzione della popolazione parassitaria nell'animale ospite con una conseguente riduzione dell'uso di farmaci.

Controllo integrato.....

Controllo biologico

1. Animali che si nutrono di ospiti intermedi e vettori: uccelli acquatici e terrestri
2. Invertebrati che si nutrono degli stadi a vita libera dei parassiti
3. Batteri patogeni
4. Funghi patogeni o che si nutrono degli stadi a vita libera dei parassiti

Controllo integrato.....



(*Duddingtonia flagrans*): si nutre di larve di nematodi presenti nell'ambiente

Questo fungo presenta alcuni attributi molto importanti:

1. Gastro-resistenza delle spore
2. Cresce rapidamente nelle feci fresche
3. È un nematofago molto vorace
4. È possibile produrre in laboratorio grandi quantità di spore di questo fungo
5. Studi sperimentali a lungo termine hanno dimostrato che il fungo non ha effetti negativi sull'ambiente

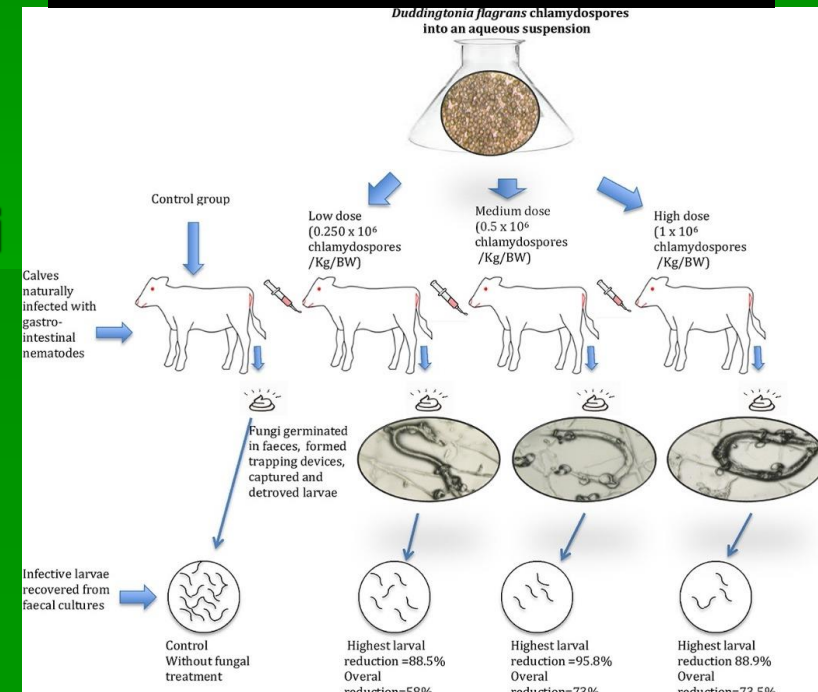
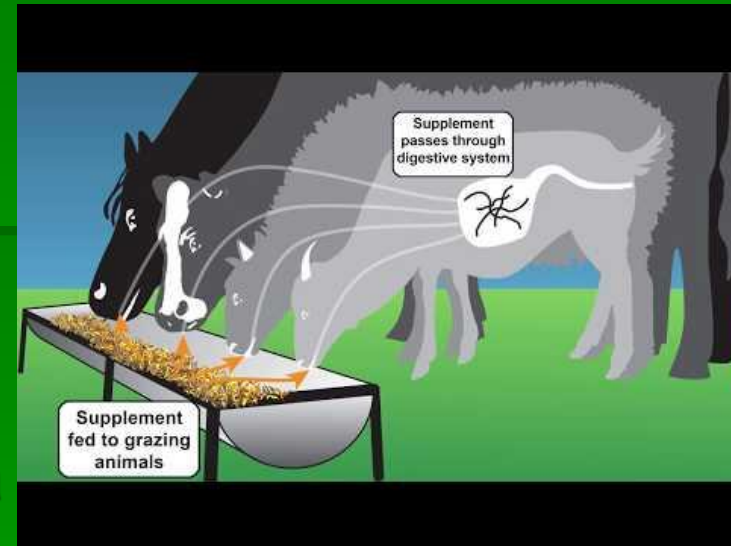
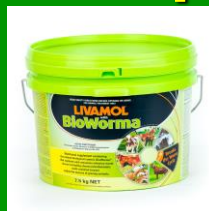


Controllo integrato.....

(*Duddingtonia flagrans*): si nutre di larve di nematodi presenti nell'ambiente

1. E' commercializzato sottoforma di additivi alimentari da somministrare per periodi abbastanza lunghi, corrispondenti ai periodi i maggiore presenza di uova nei pascoli (almeno due mesi)

È commercializzato in alcuni paesi nord-europei



Controllo integrato.....

Vaccini

- Molte difficoltà riguardo i vaccini per i nematodi e molti altri parassiti per le difficoltà di produrre quantità “commerciali” delle frazioni antigeniche e per la complessità della risposta immunitaria dell’ospite.
- Oggi però in commercio un vaccino contro le infestazioni da *Haemonchus contortus* nelle pecore commercializzato in Australia (Barbervax®)
- The vaccine contains a small dose (5 µg) of glycoproteins isolated from the intestinal cells of the parasite.

Special Issue Review

Cite this article: Vercruysse J, Charlier J, Van Dijk J, Morgan ER, Geary T, von Samson-Himmelstjerna G, Claerebout E (2018). Control of helminth ruminant infections by 2030. *Parasitology* **145**, 1655–1664. <https://doi.org/10.1017/S003118201700227X>

Received: 26 September 2017

Accepted: 20 November 2017

First published online: 8 February 2018

Key words:

Control; helminths; ruminants

Author for correspondence:

Jozef Vercruysse, E-mail: jozef.vercruysse@ugent.be

Control of helminth ruminant infections by 2030

Jozef Vercruysse¹, Johannes Charlier², Jan Van Dijk³, Eric R. Morgan⁴, Tim Geary⁵, Georg von Samson-Himmelstjerna⁶ and Edwin Claerebout¹

¹Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium; ²Kreavet, Hendrik Mertensstraat 17, 9150 Kruibeke, Belgium; ³Centre for Preventive Medicine, Animal Health Trust, Lanwades Park, Kentford, Newmarket, Suffolk, CB8 7UU, UK; ⁴Institute for Global Food Security, Queen's University Belfast, Belfast, BT9 7BL, UK; ⁵Institute of Parasitology, McGill University, Ste-Anne-de-Bellevue, QC H9X 3V9, Canada and ⁶Freie Universität Berlin, Institute for Parasitology and Tropical Veterinary Medicine, Robert-von-Ostertag-Str. 7-13, 14163 Berlin, Germany

Abstract

Helminth infections have large negative impacts on production efficiency in ruminant farming systems worldwide, and their effective management is essential if livestock production is to increase to meet future human needs for dietary protein. The control of helminths relies heavily on routine use of chemotherapeutics, but this approach is unsustainable as resistance to anthelmintic drugs is widespread and increasing. At the same time, infection patterns are being altered by changes in climate, land-use and farming practices. Future farms will need to adopt more efficient, robust and sustainable control methods, integrating ongoing scientific advances. Here, we present a vision of helminth control in farmed ruminants by 2030, bringing to bear progress in: (1) diagnostic tools, (2) innovative control approaches based on vaccines and selective breeding, (3) anthelmintics, by sustainable use of existing products and potentially new compounds, and (4) rational integration of future control practices. In this review, we identify the technical advances that we believe will place new tools in the hands of animal health decision makers in 2030, to enhance their options for control and allow them to achieve a more integrated and sustainable approach to helminth control in support of animal welfare and production.

Piccoli strongili broncopolmonari ovicaprini

Controllo

**Inutile la profilassi ambientale basata sulla
rotazione dei pascoli;**

**Impraticabile la lotta nei confronti dei
molluschi ospiti intermedi;**

**Il controllo si basa sul controllo delle altre
parassitosi e delle altre patologie**

Trichuris spp.

Controllo

- Igiene dei ricoveri e degli ambienti frequentati dagli animali
- Le uova larvate sono altamente resistenti ai detergenti e disinfettanti, quindi importante è il lavaggio
- Controllo animali di nuova introduzione