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Effects of artemisinin and *Artemisia annua* extracts on xenic bacteria isolated from clonal cultures of *Histomonas meleagridis.*

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Conclusion

- **No antibacterial effect was noticed with compound concentrations identical to the antihistomonal screening.**
- **Since no antibacterial effects were observed on the bacteria isolated from the xenic flora of six clonal *H. meleagridis* cultures the observed inhibition of histomonal multiplication is regarded as directly antihistomonal.**
- **The potential of these materials on histomonosis was subsequently tested *in vivo* in chickens and in turkeys without success.**

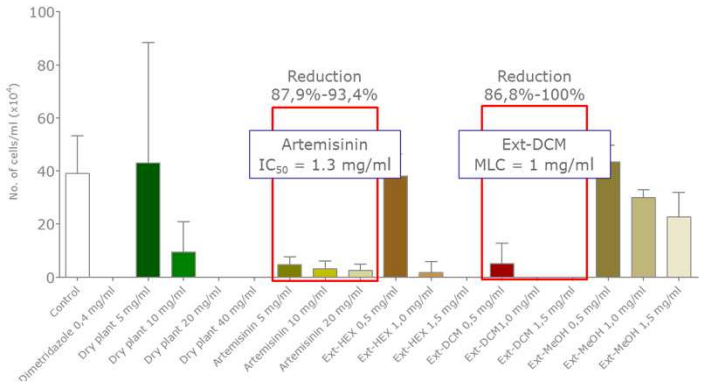
Background

Infection with the protozoa *Histomonas meleagridis* in poultry has re-emerged since the ban of effective drugs (7). Consequently efforts are set to find alternatives to chemotherapeutics to combat histomonosis. At present histomonads need accompanying bacteria when cultured in vitro, probably serving nutrient supply due to their appearance in parasitic food vacuoles. However, the relationship of the parasite and the bacteria is not fully clear.
Six previously established clonal cultures of *H. meleagridis* (5) were used to evaluate the effect of five *Artemisia annua* derived materials (i.e. dry leaves, artemisinin; and hexane, dichloromethane or methanol extracts). Dry leaves, artemisinin, hexane and dichloromethane extract displayed significant dose dependant inhibitory activity against all six mono-eukaryotic cultures (Figure 1).

Aim

The aim was to assess whether the observed inhibitory effects on *H. meleagridis* multiplication could be accounted as direct or indirect.

Figure1: *In vitro* responses of *Histomonas meleagridis* clone 6b to *Artemisia annua* dry leaves, artemisinin; and hexane, dichloromethane or methanol extracts of *Artemisia annua*



Methodology

***Artemisia annua* compounds.**

- Dry leaves from *Artemisia annua*, artemisinin (purity >99%), crude essential oil fractions from *A. annua* leaves (Ext-oil-HEX; Ext-oil-DCM; and Ext-oil-MeOH), made using hexane, dichloromethane or methanol as extraction methods.

Isolation and sensitivity testing of xenic bacteria.

- Bacteria present in the same mono-eukaryotic *Histomonas* cultures as in the antiprotozoal setting were isolated using selective media and biochemical characterisation methods.
- The antibacterial activity was assessed using the disc diffusion method (1;2). Preparation of inoculum followed the CLSI Direct Colony Suspension Method (2).
- A volume of 20 µl of the test solutions in concentrations identical to those in the antiprotozoal assay were loaded onto empty Sensi-discs. Negative controls were loaded with 20 µl PBS and positive controls contained 10 µg.

Discoveries

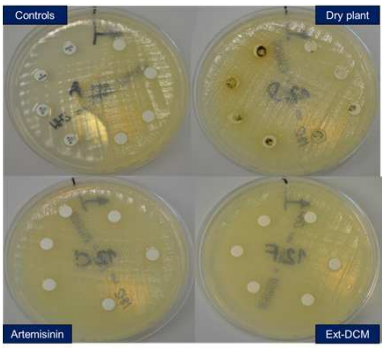
- In total 19 bacterial strains were isolated from the six mono-eukaryotic *H. meleagridis* cultures. *E.* coli (8/19) was isolated at least once from all six H. meleagridis cultures, including four APEC isolates (O1, O2, or O78). *Streptococcus* spp. (5/19) or *Proteus* spp. (5/19) were isolated from four protozoal cultures. *Staphylococcus* sp. was isolated once.

Table 1. Bacteria isolated from the different cloned cultures of *H. meleagridis*.

Cloned cultures	Bacterial isolates
Histomonas meleagridis/Turkey/Austria/2922-C6/04	*Streptococcus* sp
	E. coli O1, mucoid
	E. coli, mucoid
Histomonas meleagridis/Chicken/ Hungary/5009-C2/05	*Proteus* sp
	E. coli, mucoid
Histomonas meleagridis/Turkey/Austria/5642-C4/05	*Proteus* sp
	Proteus sp
	E. coli O2, mucoid
	Streptococcus sp
Histomonas meleagridis/Chicken/Austria/8175-C7/06	*Streptococcus* sp
	Streptococcus sp, Haem.
	E. coli O78, mucoid
Histomonas meleagridis/Turkey/Austria/2877-C3/05	*Streptococcus* sp
	Proteus sp
	E. coli O2, mucoid
Histomonas meleagridis/Turkey/Germany/4114-C18/05	*Proteus* sp
	E. coli, mucoid
	E. coli
	Staphylococcus sp

- No inhibitory effect of any of the compounds (dry plant; artemisinin; Ext-oil-HEX; Ext-oil-DCM; and Ext-oil-MeOH) was observed in any of the 19 isolated bacterial strains from any of the six investigated histomonal clones (see example on Figure 2) .

Figure2: Inhibition zones of mucoid *E. coli* O78



Discussion

The present susceptibility testing at compound concentrations as used in the antihistomonal setup revealed no inhibitory effect on bacterial growth when treated with dried *A. annua* leaves, artemisinin or either of three extractions.
It is known that artemether, a derivative of artemisinin, has no antibacterial effect on human hospital strains of *E. coli* and *S. aureus* (4). Similar investigations found that artemisinin had no antibacterial effect on *S. aureus* (3;9). However, artemisinin showed antibacterial properties at 1 mg/ml against *E. coli, E. coli* NCTC 9002 and *Proteus vulgaris* (3). In our study, the amount of artemisinin loaded onto the discs ranged between 100-300 µg/disc (20 µl of each test solution per disc) which had no antibacterial effect on the bacterial strains isolated from the clonal histomonal cultures. This is in agreement with a study where no antibacterial effect of 100 µg/disc artemisinin was found on *E. coli* or *S. aureus* (8).
To the best of our knowledge, only a single study has addressed the antibacterial effect of essential oil components extracted from *A. annua* (6) in which no inhibitory effect on *E. coli* and *S. aureus* was shown, whereas complete inhibition was obtained for *Enterococcus hirae* at 0.1 mg/ml.
Combining the results of the antiprotozoal screening with the antibacterial tests, it is reasonable to assume that the observed inhibitory effect of dried *A. annua* leaves, artemisinin, Ext-HEX and Ext-DCM, is attributed to a direct effect on histomonads and could be regarded as antihistomonal.
Ext-DCM and artemisinin were found to have the strongest antihistomonal effect in the *in vitro* studies and were therefore selected for further *in vivo* testing. Despite promising *in vitro* properties no effect on experimental *H. meleagridis* infection could be demonstrated.

Contact
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