

Role of Neem in Animal Health

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Animal diseases and veterinary public-health (VPH) problems constitute a major constraint to livestock production and safe utilization of animal products worldwide. Neem derived compounds (NDCs) can play a role in the field of animal health. One must distinguish between two levels of intervention. On the one hand for developing countries where serious socio-economic consequences, which include production losses, loss of livelihoods, poverty, food insecurity, restriction of marketing opportunities, disincentives to investment and public-health risks are tightly linked to animal diseases on the one hand and on the other hand organic livestock production and pet animals in the developed world. The most vulnerable groups, in developing countries, for whom animal diseases are particularly devastating, are poor livestock farmers and farming communities where animal diseases pose crucial constraints for the enhancement of livestock-production systems. In developed countries organic livestock production is a means of food production with a large number of rules directed towards a high status of animal welfare, care for the environment, restricted use of medical drugs and the production of a healthy product without residues (pesticides or medical drugs). Furthermore, in high-income countries, a very florid veterinary product market exists for “affection animals” (dogs, cats, horses, birds, etc... etc...).

Historically NDCs already have a consolidated use in agriculture both as bio-pesticides as well as natural biocides due to the anti-feedant properties, inhibition of growth and reproductive capacity, observed in about 400 species of harmful insects. At the same time, these substances are highly biodegradable and not harmful to the health of mammals.

The use of NDCs in different aspects of animal health such as control of ecto-and endo-parasites (lice, and ticks, gastrointestinal worms), plasmodial diseases and wound healing will be discussed.

Introduction:

Within the animal health field the problems faced are many and varied. These go from ecto-parasite and endo-parasite control to many other vector caused diseases (viruses, bacteria, monocellular (plasmodial), Arthropoda (Acari and Insecta)) and also wound healing. Each one of them taken on its own is worthy of a review. As in this short space an exhaustive treatise is not possible for each of them an attempt will be made to highlight the most important fields where NDCs can play an important role or where the results, at best, are still doubtful.

Ecto-parasites:

A) Ticks

Ticks are bloodsucking parasites of many warm blooded animals and can be transmission agents of infectious diseases (vectors) of different aetiological agents for both humans as well as for production animals and pets. Transmitted diseases are: Babesiosis, Rickettsia (typhus, rickettsial pox, Boutonneuse fever, African tick bite fever, Rocky Mountain spotted fever, Flinders Island spotted

fever and Queensland tick typhus (Australian Tick Typhus)); Lyme disease (Borreliosis) in particular by *Ixodes ricinus*, encephalitis transmitted by ticks (or TBE) and Tularaemia (rabbit fever).

The evolution of resistance of ticks (Superfamily: Ixodoidea) to synthetic acaricides has given rise to the need for new scientific investigations on alternative ways to control them (1, 2). In this regard, various studies on plants have been developed in an attempt to find extracts with acaricidal properties. There are many advantages from using plant extracts: for example, they can be used in organic cattle farming or even replace synthetic acaricides and they are associated with lower environmental and food contamination, slower development of resistance and lower toxicity to animals and humans.

Several scientific studies have appeared on the use of NDCs (aqueous and ethanolic extracts) from neem seeds or neem oil both **in vitro** (3, 4, 5) as **in vivo** (6, 7, 8). **In vitro**, both the oil and the polar extracts (NeemAzal F[®] or ethanolic extracts from seeds) were found to be active against larval stages and adults (*Boophilus microplus*, *Hyalomma excavatum anatolicum* (Ixodidae)), with regard to egg development. The obtained control on the population of ticks is comparable to that reached with products currently on the market although by a completely different "mode of action" (MoA). In fact, instead of getting a "knock down" effect the control of the pest population is achieved through sterilization and disturbance of the developmental process from larval to adult stage. Pure Azadirachtin, up to 5000 mg liter⁻¹, had no effect on egg production or the ability to feed of *Hyalomma dromedarii* (Ixodidae) adults (antifeedancy), however, at a dose of 2500 mg liter⁻¹ an anti-feedant effect was observed on larvae (8). Neem seed extracts containing 10.000 ppm of azadirachtin and other limonoids had deleterious action on the reproduction of *R. microplus* females (5)

The only study made of the repellent effect of neem oil is by Samira S. Garbouei et al. with field trials against *Ixodes ricinus* (Ixodidae) (9). The result is positive (60 - 80% repellency), even if the duration is less extended than other commercial products tested.

An alternative approach to tick control consists in supplementing the diet of the host animals with biocidal compounds. This way the blood-feeding ticks come into direct contact, through engorgement, with these compounds. The potential of neem oil, two neem oil-based liquid formulations and powdered neem seed mixed with diet ingested by goats were evaluated for their effects on *Amblyomma variegatum* ticks. The test materials at high concentrations **in vitro** induced significant mortality of immature stages of *A. variegatum* and to a lesser degree of adults. Mortality increased with the increasing concentration of the Neem products as well as Azadirachtin content. *A. variegatum* ticks that were allowed to engorge on goats feeding on a diet containing powdered neem seed in 3:1 or 2:1 proportions exhibited a significant reduction in their ability to attach and engorge. Feeding, moulting and overall development periods were also significantly prolonged. Mortalities were also higher in all tick instars and fecundity was significantly reduced. All these effects were concentration dependent. (10)

Another study describes results about the action of aqueous extracts of neem leaves (*Azadirachta indica*) on the vitellogenesis of *Rhipicephalus sanguineus* ticks, proving that these extracts in 10 and 20% concentrations do not have the potential to kill the females; however, in lower concentrations (10%) provokes

great morphological alterations in germinative cells such as the emergence of extended cytoplasmic vacuolization areas as well as the fragmentation of the germinal vesicle, even in those oocytes which were in initial stages of development (I–III), showing that neem is a potent agent which acts impeding one of the main metabolic stages of the ticks, i.e., the reproduction (11).

Considering all these different results, representing diverse neem extracts and/or formulations, different tick species, different stages of the tick lifecycle targeted, different host animals (cows, goats, camels, rabbits etc...), **in vitro** and/or **in vivo** experimental systems, different way of treating the ticks (contact or feeding) one can -all the same- make some conclusions. Neem derived compounds are active for obtaining control over tick populations but optimisation of dose, attack method, formulations, standardization etc... are still necessary before one has a reliable alternative product to synthetic acaricides already present on the market. It is also true that resistance to synthetic acaricides is on the rise so an alternative product/method will be very welcome, especially if it is less toxic both towards the treated animals as towards the operative personnel and it is also environmentally sound. Neem based products certainly are serious contenders for such a role.

B) Lice:

Sucking lice infestations in human (*Pediculus humanus capitis*) and chewing/biting lice in farm animals and pets (e.g. *Damalinia* spp) pose different problems depending on the host. In human head lice are more a social than a health care problem, while in animal husbandry, especially sheep and goats, the problem is directly related to animal health and to the quality fibre production. In recent years there has been a steady increase of the resistance of lice to products in common use, both synthetic and natural in origin (12, 13, 14, 15). It is therefore urgent to develop products with low toxicity able to control lice infestations without inducing resistance.

At ENEA research has been carried out for controlling lice both in human as in production animals (sheep and Angora goats) using highly purified extracts of neem (NeemAzal®). The data obtained for both application fields (animal and human) (16) show a good biocidal action towards the different species of lice with a protection in Angora that lasts up to 4 months after treatment.

Similar results have been described in two papers that used a methanolic neem seed extracts (17, 18). In the work of Heath et al. (17) an extract containing 1000 ppm of Azadirachtin A was used and the evolution of the population of *Damalinia ovis* up to 7 weeks post-treatment was monitored. A reduction between 85 and 99.6%, starting from the second week until the seventh, was observed. Unfortunately they did not extend monitoring for more time. Guerrini (18) has tested a methanol extract with Azadirachtin A concentrations ranging from 40 to 1250 ppm and found a clear dose effect relationship.

Considering our data (16) and those of the other two research groups, it can be concluded that the NDCs containing Azadirachtin A, are effective for the control of infestation by biting lice (*D. limbata*, *D. ovis*) and that the MoA is different from that shown by synthetic insecticides. The latter in fact have a marked "knock-down" effect, but the protection is significant only during the first month post-treatment. Also for human head lice (sucking lice) NDCs are effective (19; 20) and now a days different commercial products are present on the market. The NDCs instead show a blander initial effect that already after two weeks reaches

the same effectiveness as the synthetic products used as positive controls and, furthermore, produces a lasting effect that is maintained for 18 weeks post-treatment.

C) Fleas:

There are only two published studies using respectively methanol extract and aqueous extract for the control of flea infestation. In both cases the first indications were encouraging (21; 22).

Endo parasites:

The development of resistance to synthetic anthelmintics, associated with a high cost of medicines has limited gastrointestinal parasitic worm control in sheep and goats, fostering an interest in studying medicinal plants as alternative anthelmintics (23). The first **in vivo** studies with neem leaves (24) gave positive results. Studies made at ENEA, both **in vivo** and **in vitro**, have not been able to confirm the anthelmintic activity of extracts of neem (both leaves and seed extracts and neem oil) against *Haemonchus contortus* and *Trichostrongylus* spp. both **in vitro** as **in vivo** (25). Subsequent studies, made with more carefully designed experimental conditions, have disproven the anthelmintic activity, both **in vivo** and **in vitro** when using neem leaf and/or seed (26, 27, 28). Iqbal et al. (29) report of feeding crude neem seed powder (CP), crude aqueous (CAE) and crude methanolic extracts (CME) to sheep. Both the CP and CME demonstrated to have some effect on the EPG (eggs/gram faecal weight), corresponding to a low anthelmintic effect when compared to commercially available allopathic medicine.

Only in the particular case of strongyle L3 larvae (sheathless “host seeking” larval phase), anthelmintic activity was demonstrated by neem extracts (30). Another supportive article of an anthelmintic effect of NDCs is from Costa et al. (31) where ethanolic extract from neem seeds showed to have a strong effect, **in vitro**, on the egg hatching capabilities. The effect on the larval development stage was lesser and not comparable to the effect obtained with Ivermectin.

So the effectiveness of neem against gastrointestinal strongyls (nematode worms), which is frequently claimed, is still a moot point. Most evidence points against NDCs being an effective anthelmintic, both **in vitro** as **in vivo**, but at the same time some serious scientific evidence exists that indicates that some effectiveness subsists. Seen the complexity of the nematodes lifecycles and the various stages they go through, it might be possible to identify the exact moment in which susceptibility is effective. This still not means a cheap and easy to apply product can be developed.

Protozoan diseases:

Trypanosomiasis is an important protozoan disease of domestic animals and man. Human African trypanosomiasis (HAT) is caused by the tsetse fly-transmitted hemo-flagellates *Trypanosoma brucei rhodesiense* (in East and Southern Africa) and *T. b. gambiense* (in West and Central Africa), while animal trypanosomiasis is caused by *T. b. brucei*, *T. vivax* and *T. congolense* (32). Sleeping sickness has been on the rise in recent years and is viewed as a major health problem in many African countries, with sixty million people being at risk of infection in sub-Saharan Africa (33). The loss in livestock production and mixed agriculture alone is valued at 5 billion USD yearly in Africa (34).

Only a few studies have been carried out studying NDCs antiplasmodial activity. Ngure et al. describe an in vivo experiment to determine the anti-trypanosomal effect of aqueous extracts of the neem bark in *Trypanosoma brucei rhodesiense*-infected mice (35). The ability to control parasitaemia level, PCV (Packed Cell Volume) and also to extend the survival period of infected mice was dose-dependent, indicating that neem bark extracts contains anti-trypanosomal compounds. Of interest was the fact that the 1000mg/kg bwt neem extract was able to delay the appearance of parasites in the blood following infection, control parasitaemia levels, weight loss and also extend the lifespan of the mice with comparable ability to suramin, a known trypanocidal drug. Another study (36) using purified tetranortriterpenoids from *Toona ciliata* (fam. Meliaceae) roots also gives a clear indication that limonoids are active against *Trypanosoma*. Crude methanolic and chloroform extracts of *T.ciliata* exhibited significant antitrypanosomal in vitro activities against *T. b. rhodesiense* procyclic forms whose MIC values are 6.95 ± 0 and 43.2 ± 0 $\mu\text{g/ml}$, respectively. The two isolated compounds had lower activities suggesting that there are other antitrypanosomal compounds present or possible synergistic blend effects. Chloroform extracts were more potent than the methanol extracts, indicating medium polarity.

Omoju et al. studied neem leaf extracts used in combination with a known antitrypanosomal drug diminazene diacetate (DDA) in rats (37). They established that the combination of 125 mg/kg bw neem leaf extract combined with 7 mg/kg DDA is very effective in the treatment of trypanosomosis caused by *T. brucei brucei*, as it quickly cleared the parasites and prevented relapse infection. These researchers suggested from the deductions of this study that neem enhanced diminazene in producing its trypanocidal activity in at least three possible different ways: potentiation, synergy and increase in half-life.

Habila et al. (38) worked with Neem Seed Methanolic Extracts (NSME) on trypanosomosis caused by *Trypanosoma evansi* which is the most widely distributed of the pathogenic animal Trypanosomosis and are transmitted by biting flies, which have acquired their infection from animals harbouring the pathogenic parasites. These authors find that it appears that NSME possesses "trypanoprophylactic" potency more than trypanocidal.

So overall, the research dedicated to plasmodial diseases (malaria has not been discussed here but similar results have been obtained) certainly indicates that different NDCs have varied effects on the survival within the host of the plasmodial agent. Much more research will have to be dedicated to this very interesting application before a complete understanding of the action is understood which then could form the basis for a commercial product.

Wound healing:

When managing wounds in animal healthcare complications due to infection (bacteria), infestation (myiasigenic flies) and/or impaired healing processes (equine keloid) are often present. In the presence of complications different allopathic remedies must be applied in combination, some of which are also tissue damaging (e.g. disinfectants or antibiotic remedies) thus hampering the healing process. Veterinary wound management would be greatly facilitated if a wound dressing combining antibiotic, repellent, biocidal and healing properties, without being tissue damaging, existed. In this presentation results regarding a

herbal combination, consisting of Neem oil (*Azadirachta indica* (A. Juss)) (Azadirachtin 300 – 500 ppm) and St. John wort (*Hypericum perforatum* L.) oleum extract (Hyperforin 100 – 300 ppm) seems to combine all these necessary effects (Patent: WO2006013607) (39). This wound healing remedy is commercialised under the name of 1 PRIMARY WOUND DRESSING® by the Swiss company Phytoceuticals AG. In human already some publications exist which describe the use of this wound healing remedy both in acute as well as in chronic wounds (40; 41) where it promotes wound cleansing and granulation tissue formation.

Results will be presented which illustrate the application of this novel wound healing dressing both in sheep as in horse. The anti-myiasigenic effect permits to treat animals in the field without having to apply wound protecting medical devices such as bandaging which is often problematic due to wound localisation. The single components (neem oil or Oleolite of Hypericum) on their own don't demonstrate to have the same range of effects that one obtains in combination and which are fundamental for fostering a proper wound healing process.

So, although wound healing in animals can be problematic and an all encompassing effective treatment is still lacking, the proposed NDCs based wound healing dressing can represent a simple to apply "all in one" solution.

Conclusion

Applications of NDCs in bio-control of crop pests presents a complex picture due to the many different neem extracts available and due to the often complex lifecycles of many of the targeted pests. The same is true for the application of NDCs as a biocidal. Once more the necessity to standardise the neem extracts (**starting material** (seeds, leaf, bark, root) and **how** they are extracted (ethanol, methanol, chloroform etc...)) and how they are formulated and applied becomes important. It is without doubt that NDCs have very interesting properties - effective towards the target organism while environmentally sound and harmless to non-target organisms- that can find valid commercial applications in the field of animal health but it will call for further collaboration between the scientific community and industry including also the regulatory bodies.

Bibliography:

- 1) Ghosh S, Azhahianambia P and Yadavb MP "Upcoming and future strategies of tick control: a review" J Vect Borne Dis (2007), 44:79-89
- 2) Kiss T, Cadar D, Spînu M "Tick prevention at a crossroad: New and renewed solutions" Vet Parasitol. (2012); 187(3-4):357-66.
- 3) Abdel-Shafy S, Zayed AA "In vitro acaricidal effect of plant extract of neem seed oil (*Azadirachta indica*) on egg, immature, and adult stages of *Hyalomma anatolicum excavatum* (Ixodoidea: Ixodidae) Vet Parasitol. (2002) 106: 89-96;
- 4) Valente M et al "Effectiveness of *Azadirachta indica* watery extract on the control of bovine ticks *Boophilus microplus*" Arq. Bras. Med. Vet. Zootec., (2007) 59(5):1341-1343
- 5) Giglioti R et al. "In vitro acaricidal activity of neem (*Azadirachta indica*) seed extracts with known azadirachtin concentrations against *Rhipicephalus microplus*" Vet Parasit. (2011) 181:309-15
- 6) Rahul Srivastava S et al. "Efficacy of *Azadirachta indica* extracts against *Boophilus microplus*" Parasitol Res (2008) 104:149-15;

- 7) Webb and David M “The efficacy of neem seed extract (*Azadirachta indica*) to control tick infestation in Tswana, Simmentaler and Brahman cattle” South African Journal of Animal Science (2002), 32 (1);
- 8) DiefAlla H Al-Rajhy et al. “Acaracidal effects of cardiac glycosides, azadirachtin and neem oil against the camel tick” Pest Manag Sci (2003) 59:1250–1254
- 9) Garbouli SS, Jaenson TGT, Paolsson S “Repellency of MyggA_ Natural spray (para-menthane-3,8-diol) and RB86 (neem oil) against the tick *Ixodes ricinus* (Acari: Ixodidae) in the field in east-central Sweden” Exp Appl Acarol (2006) 40:271–277
- 10) Kaaya GP et al. “The potential of neem products for control of economically-important African ticks” Biosciences Biotechnology Research Asia, (2007) 4(1):pp. 95-104.
- 11) Denardi SE, Bechara GH, de Oliveira PR, Camargo Mathias MI “Inhibitory Action of Neem Aqueous Extract (*Azadirachta indica* A. Juss) on the Vitellogenesis of *Rhipicephalus sanguineus* (Latreille, 1806) (Acari: Ixodidae) Ticks” Microsc Res Tech (2011) 74(10):889-99
- 12) Burgess IF, “Human lice and their control” Annu. Rev. Entomol. (2004). 49:457–81.
- 13) Levot G, “Resistance and the control of lice on humans and production animals” Int J Parasitol (2000) 30: 291-297.
- 14) Martin PJ, “The development of high synthetic pyrethroid resistance in *Bovicola (Damalinia) ovis* and the implications for resistance management” Australian Veterinary Journal, (1993) 70(6): 209–211.
- 15) Meinking TL “Clinical update on resistance and treatment of *Pediculus capitis*” The American Journal of Managed Care (2004) 10, n° 9 Supl: S264–S268
- 16) Hableutzal A, Carnevali F, Lucantoni L, Grana L, Attili AR, Archilei F, Antonini M, Valbonesi A, Abbadessa V, Esposito F, van der Esch SA. “Impact of the botanical insecticide NeemAzal® on survival and reproduction of the biting louse *Damalinia limbata* on Angora goats” Vet Parasitol (2007) 144: 328–337.
- 17) Heath, AC, Lampkin, N, Jowett, JH, “Evaluation of non-conventional treatments for control of the biting louse (*Bovicola bovis*) in sheep” Med. Vet. Entomol. (1995) 4:407–412.
- 18) Guerrini, VH., Effect of azadirachtin on *Damalinia ovis* in sheep. Online J. Vet. Res. (2000) 4 (3):133–138.
- 19) Heukelbach J, Oliveira FAS, Speare R, “A new shampoo based on neem (*Azadirachta indica*) is highly effective against head lice in vitro” Parasitol Res (2006) 99: 353–356.
- 20) Abdel-Ghaffar F, Semmler M, “Efficacy of neem seed extract shampoo on head lice of naturally infected humans in Egypt” Parasitol Res (2006).
- 21) Guerrini VH and Kriticos CM “Effects of azadirachtin on *Ctenocephalides felis* in the dog and the cat” Vet Parasitol (1998) 74, Issues 2-4, 31:289-297.
- 22) Kilonzo BS, Ngomuo AJ, Sabuni CA, Mgode GF, “Effects of *Azadirachta indica* (neem) extract on livestock fleas in morogoro district, Tanzania” Insect Science and its Application (2001) 21, Issue 1:89-92.
- 23) Perry BD and Randolph TF “Improving the assessment of the economic impact of parasitic diseases and of their control in production animals” Vet. Parasitol. (1999) 84:145-168

- 24) Pietrosemoli S et al. "Empleo de hojas de Neem (*Azadirachta indica* A. Juss) en control de nematodos gastrointestinales de bovinos a pastoreo" Rev. Fac. Agron (LUZ). (1999) 16 Suppl 1:220 – 225.
- 25) van der Esch SA, Carnevali F, Amici A. "Effect of Neem derived products on Gastro-intestinal Nematodes **in vitro** and **in vivo**" Workshop Biological Control of Plant, Medical and Veterinary Pests. Wetzlar, Germany (15-16 November 2004)
- 26) Costa CTC et al. "Anthelmintic activity of *Azadirachta indica* (A. Juss) against sheep gastrointestinal nematodes" Veterinary Parasitology (2006) 137:306–310.
- 27) Hordegen P et al. "The anthelmintic efficacy of five plant products against gastrointestinal trichostrongylids in artificially infected lambs" Vet Parasitol (2003) 117:51–60.
- 28) Worku M, Franco R, Miller JH "Evaluation of the activity of plant extracts in Boer goats" Am J Anim Vet Sci (2009) 4(4): 72-79
- 29) Iqbal et al. "*In vivo* anthelmintic activity of *Azadirachta indica* A. Juss seeds against gastrointestinal nematodes of sheep" Vet Parasitol (2010) 168 (3-4):342-5
- 30) Hordegen P et al. "In vitro screening of six anthelmintic plant products against larval *Haemonchus contortus* with a modified methyl-thiazolyl-tetrazolium reduction assay" Journal of Ethnopharmacology (2006) 108(1):85 - 89
- 31) Costa CTC et al "*In vitro* ovicidal and larvicidal activity of *Azadirachta indica* extracts on *Haemonchus contortus*" Small Ruminant Research 74 (2008) 284–287
- 32) Igbokwe IO, "Dyserythropoiesis in animal Trypanosomiasis" Rev d'Elevage et de Med Vet des pays Trop 1989; 42: 423-429.
- 33) WHO, (1998). The control and surveillance of African trypanosomiasis, Technical Report Series 881
- 34) Samdi SM, Fajinmi AO, Kalejay JO, Wayo B, Haruna MK, Yamap JE, "Prevalence of trypanosomosis in cattle at slaughter in Kaduna central abattoir" Asian J Anim Sci (2011) 5(2): 162-165
- 35) Ngure RM et al "Anti-trypanosomal effects of *Azadirachta indica* (neem) extract on *Trypanosoma brucei rhodesiense*-infected mice" Eastern J Med (2009) 2-9
- 36) Chitua M et al "Antitrypanosomal tetranotriterpenoids from *Toona ciliata* roots" Agr Biol J N America (2011) 2(7):1042-1047
- 37) Omoju VU et al "The effects of combination of methanolic leaf extract of *Azadirachta indica* and diminazene diacetate in the treatment of experimental *Trypanosoma brucei brucei* infection in rats" Asian Pac J Trop Med (2011) 337-341
- 38) Habila N, Humphrey NC, Abel AS "Trypanocidal potentials of *Azadirachta indica* seeds against *Trypanosoma evansi* Vet Parasitol (2011) 180(3-4):173-8
- 39) Carnevali F and van der Esch SA. "(WO2006013607) Composition comprising neem oil and oil extract of *Hypericum* having healing, repellent and biocidal properties for treating external wounds" WIPO Database
- 40) Läuchli S, Hafner J, Wehrmann C, Frenc LE. "Post-surgical scalp wounds with exposed bone treated with a plant-derived wound therapeutic" Journal of Wound Care, (2012) 21(5):228 – 233

41) Läuchli S. "1 Primary Wound Dressing®: clinical experience" Hospital Health Care (2012) 1:1-3 Online Journal