Fasciola hepatica: Impact on Dairy Production and Sustainable Management on Selected Farms in South Africa

(PRJ-0107-2016)

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Year 2016/2016 (January 2016 till December 2016)

Project goals

Goal 1 - Faecal sampling and worm egg counting : Monthly [Months 1-9]

Achievements

As reported before, budgetary constraints led to termination of faecal collection and worm egg counting on Farm 1-3 in May, 2016, but not on Farm 4, where it was deemed essential by the project team to continue, due to clinical fasciolosis having been encountered there in September 2015. This decision is vindicated by the faecal egg count and snail recovery results appended to this report.

It is unfortunate that the faecal worm egg counting on the other farms could not be continued with, for the reason that the snail survey is indicating lower levels of prevalence and incidence of the intermediate hosts of Fasciola, and it would have been very valuable to be able to correlate overlapping monthly results over the rainy season of 2015 and 2016.

The results of the routine counts are presented in "MelkSA Fasc-EPG+Ens'm-2016 JAAR-Vrsl'g.docx", which is appended to the report.

No Non-achievements / underperformance has been reported

Goal 2 - Faecal sampling and worm egg counting : Ad hoc (Fasciolosis outbreaks & requests by farmers for evaluation of other animal groups) [Months 1-11]

Achievements

In order to evaluate the efficacy of the anthelmintics used and the possibility of resistance of the parasites to the available anthelmintics, farmers involved in the project have been requested to collect samples of faeces ad hoc from about 15 cattle, both before and 21-30 days after treatment for *Fasciola* infection. However, despite repeated emphasis, this was not done, and now the opportunity has passed, due to the budgetary constraints mentioned. On the other hand, it is strongly to be recommended that such testing should be done, as the paucity of effective anthelmintics and the serious deleterious effect that *Fasciola* sp. has on the production of dairy cattle makes it essential that farmers test, in order to receive an early warning if repeated drenching is perhaps leading to anthelmintic resistance.

Despite the above, JvR did personally collect a total of about 40 faecal samples during

routine snail surveys from animal classes and groups not included in the project, processed them and conducted worm egg counts, and at the same time farmers were further urged to make use of this valuable, though limited, service whenever they wanted information on animals not included in the project trials.

Most important of the ad hoc samples was a set from a group of 20 calves suffering from diarrhoea on one of the farms. On the advice of a veterinarian from the CapeCrossVet practice, the calves had been treated for *Eimeria* spp. coccidiosis, and from the samples it was confirmed that two very pathogenic species of *Eimeria* present had been responsible for the diarrhoea, and that the treatment had been effective, with relatively few of the parasites having remained.

No Non-achievements / underperformance has been reported

Goal 3 - Faecal sample ELISA analysis (if practicable) [Months 10-11+]

Achievements

As stated in the second quarterly report of 2016, the laboratories which conducted ELISA testing for worm antigen in faeces ceased to do so, on account of the fact that, as reported to JvW, the tests did not give a dependable indication of the levels of *Fasciola* infection in infected animals. Furthermore, budgetary restraints preclude further investigations in this field for the foreseeable future, unless considerable additional funding can be obtained. It will also be necessary to obtain new sets of samples from infected animals.

No Non-achievements / underperformance has been reported

Goal 4 - Snail surveys (monthly) (Routine, as in 2015) [Months 1-9]

Achievements

Snail surveys: While some of the highlights of the snail survey are listed below, the details of the latest snail sampling results are reported in the following document: "MelkSA-Fasc-Slak 2016f J'r Vrsl'g-RC2-fin2017".

On each of the four farms ten samples of mud were collected in similar fashion per occasion from each of the six marshy (muddy) spots, previously selected on the strength of visual evaluation for potential to serve as reservoirs for the snails. A notable achievement in the project is that complete sampling from all selected mud spots on each trial farm, and sample processing was achieved without exception, monthly, according to the trial protocol, despite rain on a number of occasions and almost impassible muddy roads in a few instances.

The numbers of the intermediate snail hosts of *Fasciola* sp. recovered from time to time give a rough estimate of not only the potential effect the parasite could have on the production of susceptible hosts, but also of the seasonal cycling of the snails. The latter is important not only as an indication of the risk to the animals exposed to infection at any given time, but also in relation to climatic conditions such as rainfall and temperature. Hence four trial farms were selected in the Tsitsikamma region, on the strength of farmer observations on the importance of *Fasciola* sp. infection, and visits to the farms were continued throughout 2016 despite serious budgetary constraints, for routine snail recovery and evaluation.

A further reason why the project team insisted on persevering with the snail surveys over the course of the project is that the results may lead to suggestions for methods of biological control or at least large-scale reduction of the chances of contact between the final hosts (the cattle) with the infective stages of the trematode parasites, in this way to reduce the dependence on chemicals for the control of fasciolosis.

Differences between muddy spots in population by target snail species: While all the marshy spots selected appeared on visual assessment to be similarly suitable for propagation of

the intermediate hosts, it is clear from the results that spots situated very close together vary to a large extent in suitability for sustaining snail populations. This variation is of particular interest, as it may have potential for rapid and relatively low-cost evaluation of farms for suitability to population by the snail intermediate hosts of the trematode species involved, as well as for highlighting those spots which need to be specially considered when methods of biological control are developed and tested.

Sampling of snails for laboratory investigation: Whenever relatively large numbers of snails are recovered from any given spot, a few of the individuals were removed for dissection in the laboratory for the presence of immature stages of the worms involved. However, as explained below, the numbers had to be reduced to very low levels, so as to have the minimal effect on the epidemiology of the snails involved, during seasonal cycling.

Results over the present report period: No specific rainfall figures have been obtained for the duration of the project (this is to be done for further evaluation of the results of the project) but were lower than over the corresponding periods of 2016. Furthermore, irrigation has been common at times when the rainfall was inadequate. This latter was of great help regarding the recovery of snails from the mud, for the reason that the muddy patches mostly contained spots of soft mud, in contrast to the less moist, firm mud in the absence of irrigation, from which it is difficult to recover snails that happen to be present.

As can be seen from the series of graphs in the attached report, considerably smaller numbers of snails were recovered monthly during 2016 from the sampling spots on each of the four farms, than the mean monthly recovery for 2015. However, the numbers of snails recovered from Farms I, II and IV rose considerably over the latter half of 2016. Furthermore, of special importance is that, on Farms I, II and IV, where practically no snails were recovered from the sampling spots that were subjected to major earthworks for improved drainage thereof, the snails have subsequently been returning progressively over the course of 2016.

No Non-achievements / underperformance has been reported

Goal 5 - Irrigation water sampling for worm eggs (When faecal egg counts of trial animals are relatively high) [Months 10-12]

Achievements

As discussed in detail in the second quarterly project report, the aim with this part of the project is to ascertain to what extent the Fasciola sp. worm eggs in the run-off of the milking parlours gain access to the pastures of the animals via irrigation from these dams, as this could constitute an important source for infection of the intermediate hosts of *Fasciola* parasites in muddy spots on pastures irrigated with this water.

As explained previously, this is one of the novel approaches planned for future investigation as part of the later stages of the project. However, while it was envisaged for investigation during 2017, the latter budget does not allow for pursuance of this potentially valuable approach within the near future.

No Non-achievements / underperformance has been reported

Goal 6 - Worm recovery from livers of slaughtered animals (As and when trial animals slaughtered, or in outbreaks of fasciolosis) [Months 2-12]

Achievements

A very valuable set of liver samples was obtained from a drug efficacy test conducted locally. The livers of 12 cattle were homogenised and aliquots of each liver sampled and frozen for future evaluation (when funds become available) as to the numbers of worms per liver (which were recorded at the time of the post mortal processing of the liver tissue). These samples could give a

very good indication of the value of laboratory tests, particularly those dedicated commercial ELISA kits aimed at quantifying levels of *Fasciola* antigen in test samples. And, if indeed accurate and practical, it could constitute a relatively inexpensive way of evaluating the potential of different farms for harbouring the parasite and its intermediate hosts. However, due to budget constraints, this testing will perforce have to be postponed to the 2018 project year.

One further liver from one of the trial farms was also processed by the research team when a cow apparently suffering from fasciolosis was slaughtered while the team was on a monthly visit to the farm. Samples were taken and frozen together with the serum samples collected over that period from the project cattle for evaluation when the available funding makes this feasible.

No Non-achievements / underperformance has been reported

Goal 7 - Serum : Liver enzyme analysis (selected samples) (In relation to egg counts) [Months 10-12]

Achievements

Blood serum analysis for liver enzymes : This is an assay of intracellular enzymes that are set free from the liver cells and enter the hosts bloodstream during the hepatic migratory phase of the immature *Fasciola* parasite, which destroys large numbers of liver cells as it literally eats its way through the liver capsule and liver tissue over a period of some weeks, on its way to its final destination in the bile ducts.

GGT and GLDH enzyme analysis was recently launched on a batch of selected samples that is too small for meaningful interpretation of the results as yet. However, the preliminary results (included in [DOCUMENT1 - *Fasciola* Project - Goal 1: FAECAL WORM EGG COUNTS 2016].

The few samples included in the initial analysis were selected to span May-October 2015 (the only time when an episode of clinical fasciolosis was encountered on any of the farms during the course of the project) for both Farm IV (where the clinical cases of fasciolosis occurred) and "negative" Farm III. Being so small in number, the results should be regarded as of very preliminary nature, but are encouraging nonetheless, as regards apparently having been able to pinpoint the period of prime *Fasciola* sp. challenge on Farm IV. And, fortunately, funding allocated may allow the analyses to continue on a very limited scale during 2017.

No Non-achievements / underperformance has been reported

Goal 8 - Serum : Fasciola ELISA analysis (selected samples) (In relation to egg counts) [Months 10-12]

Achievements

As in some of the other goals (above), the project budget did not allow ELISA testing to be launched. Furthermore, seen against the background of (i) practically year-round presence of the *Fasciola* sp. intermediate snail hosts and thus the potential for transmission of the parasite over much of the year; (ii) the long half-life of the *Fasciola* sp. immune antibodies in the host; and (iii) the fact that their levels do not accurately reflect parasite numbers, the considered opinion is that less advantage is to be expected in relation to input from ELISA antibody assay, than from that of the liver enzymes circulating in the bloodstream. The latter, in contrast, have the potential to reflect actively migrating, immature parasites, i.e. those which cannot be detected by the conventional tests, such as faecal worm egg counts. Furthermore, the institution, calibration and confirmatory testing of the ELISA assay at the Faculty will be costly in relation to the funds available, in contrast to the liver enzyme analysis of stored serum samples when practicable, since this is a thoroughly validated, routine test at the Faculty, and considerably less costly.

No Non-achievements / underperformance has been reported

Goal 9 - Questionnaire : On-farm execution (Outsourced) [Months 3-12]

Achievements

The funds available for 2016 did not, and those of 2017 do not allow any leeway for execution of the small-scale survey. Furthermore, the survey has more bearing on the next phase of the project, i.e. the expansion of the results of the project to farms with problems with fasciolosis, than in Phase 1. Hence the survey cannot be regarded as a priority at present.

No Non-achievements / underperformance has been reported

Goal 10 - Technology transfer (Oral and written) [Months 3-12+]

Achievements

An interview of JvW with Mr Hennie Maas was broadcast on the RSG radio station, and a video'd interview made at the same time for technology transfer;
An article of JvW was published in two parts, in separate numbers of the local dairy industry periodicle.
A presentation on fasciolosis and the aims of the present project was given by JvW at the time of one of the management meetings of MILKSA in Pretoria.

No Non-achievements / underperformance has been reported

Goal 11 - Training of farm workers in snail surveying (When snails high in number) [Months 2-12+]

Achievements

On two of the trial farms stockmen helped with snail recovery from the various selected muddy spots. They also helped with the sieving that is entailed in the work, and in the process, they were informally trained as regards the parasites involved, their recovery from marshy patches and their relationship to the various species of snail. A total of about 5 workers were trained in this way, and the general level of interest was high. For example, questions are posed on the parasite-snail relationship, the effect of the parasites, and some even enquired about the scientific names of the different snail species. In the process of the training, one of the helpers suggested a shorter method than what we were using at the time for detecting snails intertwined with organic material, such as grass leaves and roots in mud samples. This greatly improved the speed of processing of such samples, something that was much welcomed, given the fact that the amount of such organic material encountered in the samples increased considerably over the period with lower rainfall. The training did not include snail identification and is probably unlikely to be included in the future, due to the fact that the identification is not straightforward, to the extent that, from time to time, the project team also needs to consult experts in the field of snail taxonomy and identification.

No Non-achievements / underperformance has been reported

Goal 12 - Soil & Grass : Sampling for detailed analysis: Snail antigen detection (Developing more convenient method for routine snail prevalence evaluation) [Months 8-10]

Achievements

In the light of the shortage of funds, as mentioned above, the soil and grass sampling for snail antigen detection could not be launched during the 2016 project

year. However, soil analysis is to be initiated in the 2017 contract year, and to proceed to the limit of the available funds.

No Non-achievements / underperformance has been reported

Goal 13 - Soil : Sample analysis (Chemical & Physical) [Months 8-11]

Achievements

In the light of the shortage of funds, as mentioned above, the soil and grass sampling for snail antigen detection could not be launched during the 2016 project

year. However, soil analysis is to be initiated in the 2017 contract year, and to proceed to the limit of the available funds.

No Non-achievements / underperformance has been reported

Goal 14 - Plant survey (marshy patches on pasture): Snail preference analysis [Months 8-11]

Achievements

In the light of the shortage of funds, as mentioned above, and in common with soil and grass analysis, it is not possible to launch the plant survey during the 2017 project year, but instead is to be conducted in 2018 or later, as and when the necessary funding may become available, and granted the availability of a project team at that time.

No Non-achievements / underperformance has been reported

Goal 15 - PCR : Soil (mud) & Grass analysis: Snail antigen (Developing more convenient method for routine snail prevalence evaluation) [Months 9-11]

Achievements

In the light of the shortage of funds, as mentioned above, it was not possible to launch the PCR analysis of soil and grass for the detection of snail antigen in 2016, since this will entail considerable develomental work in the laboratory and method validation, for which the necessary funds are not available for the project contract year, but preparatory work is to be done in 2017 for launching in 2018, granted the necessary funds.

No Non-achievements / underperformance has been reported

Goal 16 - Polymerase chain reaction (PCR) : Developing methodology in the lab: Snail antigen detection [Months 6-10]

Achievements

In the light of the shortage of funds, as mentioned above, it was not possible to develop the methodology in the laboratory in 2016 for detection of snail antigen, but preparatory work is to be done in 2017 for launching in 2018, granted the necessary funds.

No Non-achievements / underperformance has been reported

Goal 17 - Small, preliminary Fasciola management trial [Months 2-12+]

Achievements

As explained above, present constraints in funding may necessitate postponement of completion of Goal 17 to 2018, particularly as it may be necessary for local supervision of the trial to be outsourced to the resident CapeCross Veterinary Services practice. However, preparations for the trial are to be done in 2017 for launching in 2018, granted the necessary funds.

No Non-achievements / underperformance has been reported

Goal 18 - Data analysis [Months 9-12+]

Achievements

Preliminary analysis of the snail recovery and egg counting data is being done in the form of graphs for depicting the results. While these are very valuable as indication of progress made, it has not included statistical analysis to date, as this is scheduled for the latter stages of the first part of the project. Furthermore, as explained above, present constraints in funding may necessitate postponement of completion of Goal 18 to 2018.

In addition to data from the project, there is a great deal of principally milk production data from at least one of the farms, that could potentially be of great value as an adjunct to the results being obtained in the project.

Hence a continuous search is being conducted for the necessary funding for this initiative, and a partner with the necessary experience for sophisticated evaluation of such large data sets.

No Non-achievements / underperformance has been reported

Goal 19 - Preliminary, tentative recommendations for sustainable Fasciola management [Months 10-12+]

Achievements

One approach that has been in the planning stage and suggested to various persons involved

over the period, is to make use of a novel approach to strategic fencing of snail-infested marshy spots on pasture, to be able to attempt, at times of potential high levels of challenge of the cattle, to limit contamination of snail-infested marshy spots on pasture with faeces from infected cattle, in this way to break the life life cycle string of the parasite. To this end, plans have been formulated for sustainable management, and a farmer approached for institution of small-scale evaluation of the novel approach. While it has not been taken up as yet, this is to be pursued during the present project year. One stumbling block, however, is that the farmers involved in the project are so involved in the milking routine, that it is difficult even to have in-depth discussions with them during visits to the farms in relation to the running of the project.

As in the number of cases above, no further progress with the conduction of the envisaged small trial can be reported at present, also due principally to the lack of funding, which precludes the necessary developmental work and visits to the farm(s) concerned in relation to the conduction of the trial.

No Non-achievements / underperformance has been reported

Income and expenditure statement

Income and expenditure statement	Melk-SA-Finans'e JrVrsl'g-2017.xls
Unnecessary spending during period	No

Popular Report

Fasciola Proj 2016 Opsomm'e J'rVrsl'g.docx

Additional documentation

MelkSA Fasc-EPG+Ens'm-2016 JAAR Vrsl'g.docx
MelkSA-Fasc-Slak 2016f J'r Vrsl'g-RC2-JvW-fin2017.docx

Statement

Levy funds were applied only for the purposes stated in the contract	Yes
Levy funds were applied in an appropriate and accountable manner	Yes
Sufficient management and internal control systems were in place to adequately control the project and accurately account for the project expenditure	Yes
The information provided in the report is correct	Yes