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DAIRY COWS

PROTOTHECA, AN UNDERESTIMATED MASTITIS AGENT



First Piemontese's bovine infected by Prototheca (L.Midulla Agrilab 2013)

IN ITALY, AS IN THE REST OF THE WORLD, PROTOTHECA IS AND HAS OFTEN BEEN CONSIDERED AN OCCASIONAL AGENT. AN IN-DEPTH STUDY SHOWS, HOWEVER, THAT THE DISEASE IS UNDERESTIMATED AT THE LEVEL OF BOTH NUMBERS AND SEVERITY. IT IS NOW TIME FOR THAT EVERY PROFESSIONAL IN DIFFERENTIAL DIAGNOSIS - TO RECOGNIZE MASTITIS CAUSED BY PROTOTHECA.

In 1880, Kuhn and Zopf isolated for the first time the alga Prototheca, but in order to get the classification by the West it was necessary to wait until 1916. Lerche, in 1952, discovered that the alga in question was responsible for alterations of the udder in cattle, and a few years later, even the Human Medicine signaled skin diseases and gastrointestinal disorders in people, supported by the same causative agent.

After over 130 years, the topic Prototheca is still a very sensitive issue and bovine mastitis caused by it appears in Italian farms without being well recognised and often it is not known how to deal with it.

The infection worldwide can be considered ubiquitous and South America, because of its hot and humid climate, has always been considered as the area most at risk. However, in recent years, there

have been reports of mastitis caused by Prototheca in many other countries with climates theoretically less suited to its proliferation.

Until now, in many parts of Italy, but also in other parts of the world, many have considered this alga as an occasional agent, often without even attempting a research, with the result that the prevalence is likely to be underestimated and consequently the level of attention within herds.

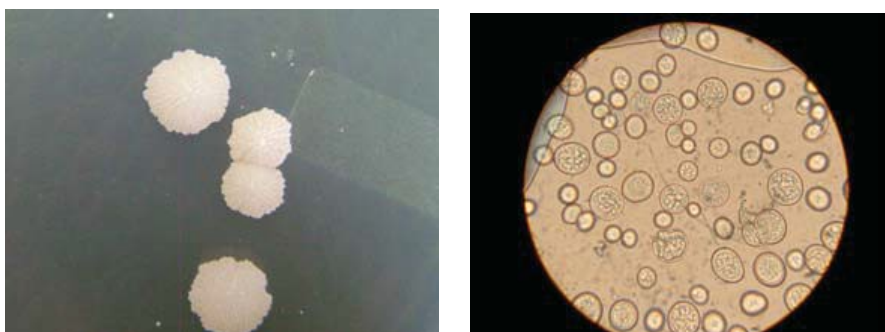
For these reasons, the laboratory Agrilab of Centallo (Cn), which deals with microbiological and chemical analysis since 1978, working in the field of food for humans and livestock, has in recent years focused on intensive research, which has allowed to investigate some issues regarding the pathogens that cause mastitis, among which is the alga Prototheca.

The laboratory has documented how, in two years, the numbers of cases of mastitis by Prototheca in Piedmont have been transformed from "academic legends" to problems of primary importance for the breeding of dairy cows, primarily as a result of a strong campaign aimed at both farmers and veterinarians. As a result it has been raised to a new awareness and a greater attention to this pathogen, which field tests have shown to be characterized by a significant impact compared to other well known pathogens.

Until a few years ago Prototheca was not well known, later it was decided to search for it only during mastitis outbreaks resistant to usual therapies, but today, thanks to the studies conducted, control in Piedmont is widespread and the search for Prototheca is placed, in terms of importance, on the same level of contagious mastitis caused by coagulase-positive staphylococci and Streptococcus agalactiae.

THE STUDY CONDUCTED IN PIEDMONT, AN INTERESTING INSIGHT

The investigations were carried out on random samples coming from 400 herds, received in Agrilab for routine analysis and control. The milk in question corresponded to about 6,000 tons produced per day, from a total of nearly 25,000 animals. The affected area covered the whole Piedmont, with an eye towards the provinces of Cuneo and Turin, characterized by a greater number of companies.



1- Colonies of Prototheca and the alga viewed at the optical microscope with their characteristic circular or oval shape (L. Midulla Agrilab 2013)

The herds in the group represented a cross-section of typically numerically diverse of Piedmont breeding stock. In fact, the herds included in the study covered both the smallest and oldest farms, where the animals are in lower set with only 15 cows in lactation and those built more recently, according to the most modern building standards, where there are more than 150 lactating cows, with peaks of 400.

The breeds were represented, in descending order, by: Friesian, Piemontese, Simmental, Alpine Brown, Jersey. Of the 400 farms studied, 51 were positive, that is, 12.7% of the total.

In 2011, when Agrilab decided to start the investigation of Prototheca, the only research performed was on those cows that were resistant to common therapies. Only in those cases the medical history would suggest a possible case of Prototheca, also indicated by the low percentages of positives reported in the area. In a second phase, however, it was decided to deal with the disease on a herd basis and not on individual cases, starting directly from bulk milk.

This approach has immediately proved to be simple, fast, inexpensive and highly specific, allowing the identification of only one positive animal in the whole herd, without neglecting the need to monitor all the cows present (as it is the case with random sampling).

The percentage of positive cows within single farms was about 10% of the animals, with cases of infections very small (1 or 2 heads only) or, vice versa, most worrying were cases with 25-30% of the animals positive (see Figures 1 and 2). A quarter of herds have registered low positive rates, with less than 5% of infected animals, however, the same proportion can be seen in herds with mastitis from Prototheca represented in the more worryingly band, seeing in the same herd, a number of positives greater than 15%.

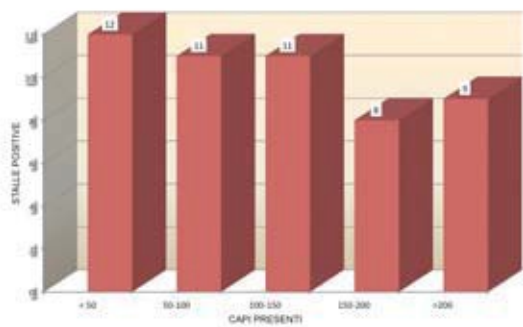


Figure 1: Relationship between the positive herds and the number of cows in lactation

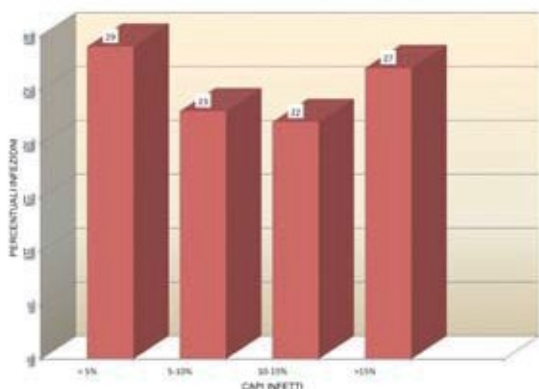


Figure 2: Percentage of infected animals for each positive herds

The study has also identified an exceptional case and perhaps unique, in which 100% of animals were positive. It was in a herd with 17 milking cows where closure was the only possible solution for the farmer. The infection, most likely, has begun to affect the cell count in 2007, with an

inexorable and irreversible progression. In this case, the delayed diagnosis was fatal for the farm. If the pathogen had been isolated in the early stages of infection, maybe today that farm would have had a different outcome.

The section of Piacenza of Institute Zooprofilattico of Lombardy and Emilia Romagna carried out the identification in 20% of positive herds and in all cases the outcome has identified *Prototheca zopfii* genotype II, in perfect concordance with the rest of Italy and the world.

DIAGNOSIS IN THE LABORATORY AND IN THE FARM

After sowing on selective medium (*Prototheca* isolation medium, Pim) and incubation for 48 hours at 37 ° C, colonies of *Prototheca* are creamy-white coloured, with a very characteristic "cauliflower" structure, rough surface and the edges just jagged. On the optical microscope algae may show a circular, oval or tile shape; the shrine is very pronounced (hence the name of the seaweed) and inside you can see a kind of granulation which corresponds to the endospores, which originate from the daughter cells. With a certain frequency it is possible to observe cells in replication and others cells at the end of this phase (see photos 1 and 2).

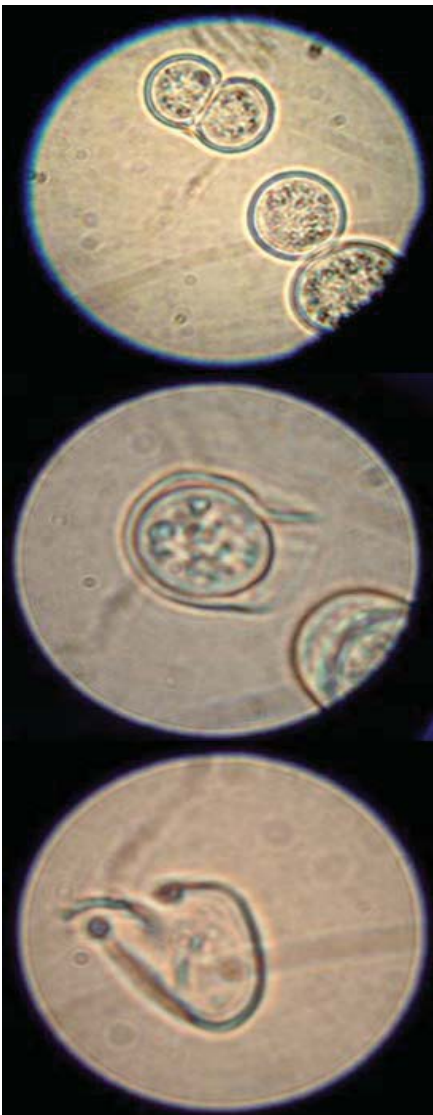


Photo 2: Prototheca cells observed at the optical microscope, ordered according to their stages of development. The first phase of replication, the second with the theca already open and the third without endospores (L.Midulla Agrilab 2013)

The report of the analysis does not arouse any major surprises in the breeders since the cows tested positive showed, in most cases, rather obvious signs. Such symptoms make it more easy to understand and to accept the result of the laboratory, creating a situation often opposite to what is found in case of, for example, positivity of coagulase-positive staphylococci.

The elements commonly distinctive are a high number of somatic cells, the presence of mastitis, that frequently relapsed, resistance to therapy, often even responsible for a worsening of the clinical picture in place, and finally one or more quarters "mysteriously" atrophied. It has been considered essential to work on two distinct fronts, laboratory and breeding, in order to investigate each case found on plate and check it immediately in the milking parlour, to adequately understand the trend of the disease.

In the bibliography, the Prototheca mastitis is defined as subclinical and chronic. The study of Agrilab, however, has found many cases in which the prevailing acute form, with swelling of the quarter concerned and increased number of cells in order of millions, could be confused by the breeders with E. coli mastitis.

In the majority of the cases, a very aggressive onset has been noticed, with the discharge of hundreds of thousands of algae per ml (up to a maximum of 6,500,000), followed by a progressive decrease. In contrast, somatic cells, after very high peaks, have settled at medium-high values and only in 20% of the cases are positioned below the 250,000 units. The infection of a positive bovine was observed both before and after the dry period, since the elimination of Prototheca is characterized by an discontinuous emission but absolutely always present.

When the milk is altered it presents a clear and watery appearance; this aspect frequently invokes the attention of the breeder (see photo 3).



Photo 3: Normal milk on the right, watery milk from Prototheca mastitis on the left (L.Midulla Agrilab 2013)

After a clinical visit, however, it may be found, by palpatory examination, tissue fibrosis and the presence of nodules on the quarter, affected that, getting chronic, undergoes atrophy and retracts gradually, taking an aspect defined "tobacco pouch", that is certainly another pathognomonic factor (see photo 4).

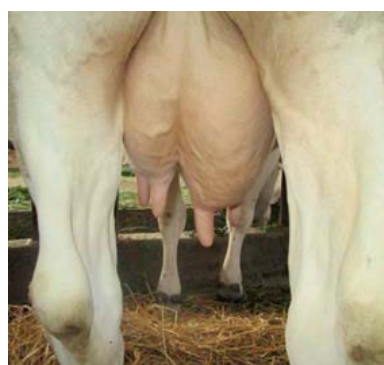


Photo 4: Cows with chronic Prototheca mastitis and atrophic quarters (L.Midulla Agrilab 2013)

As reported in literature since 2000, Prototheca tends to invade the tissue of the udder and subsequently to congest the alveoli and the ducts; the number of algae excreted, also, is not correlated with the number of those present inside the udder. In the picture n° 5 it is possible to appreciate the lymph node supermammary of a cow slaughtered as a result of an infection by Prototheca, inside which the analysis has detected the presence of the alga, while others samples were negative (mesenteric, portal, aortic, lymph nodes, tonsils).



Photo 5: Supermammary linphonode positive to the alga(L.Midulla Agrilab 2013)

PRACTICAL MANAGEMENT OF A HERD POSITIVE FOR PROTOTHECA

Screening has started with a sample taken from the milk tank and the choice of Agrilab was to investigate every single farm with one goal in mind: prevention. For this reason, the recommendations suggested to the farmer depended not only by the performance of somatic cells or by history of mastitis reported. Often, in fact, a herd considered "healthy" from the point of view of the cell count can conceal latent infections that probably have not yet had a significant effect on the tank, but, however, with time are able to infect the other animals present.

When a milk tank is found positive it is necessary to check the single lactating cow and, from then on, samples must be taken from each cow or heifer in post partum. Every cow found positive must be isolated in a separate box from other animals and milked last, since the contagion is possible in the milking parlor.

The boundary between the Prototheca mastitis classified as environmental or contagious disease is very thin and these features are fused together, resulting in the need to implement very careful and articulate management. The sample may be taken "quarter by quarter", or on a pool of quarters, a method proved to be equally reliable. In case of detection of a few colony-forming unit the process can be repeated to determine whether it is a weak infection or false positivity, which could be explained by the environmental pollution of the sample.

If the herd has a low percentage of positive cows (<5%), treatment of infected animals may be the best advice to be given to the farmer. If the percentage exceeds 10%, it should certainly be proposed a containment strategy, since it is not possible to eliminate every cow with mastitis by Prototheca. Starting from this need, Agrilab, in 2013, embarked on a new experiment, in order to propose a second solution to the more drastic option of killing the animal, represented by the drying of the quarter.

Practically, on each infected cow found, once established that the infection was only related to one of the quarters, it was proposed to acid burn the quarter itself. To date, the test was carried out on 23 animals, monitored for 7 months, and of these, only three showed positivity of another quarter, with relapsing infection by Prototheca. The other 20 cows, however, are still negative, with somatic cells less than 150,000 units and a good milk production, with some peaks of 45 liters, due to the compensatory activity of the three healthy quarters. However, it can be

still premature to claim that the method is functional or not and whether it is really averted the risk of recurrence.

In any case, we can say that, after a sufficiently significant time from the cauterization of the quarter, the cow continues to have important production of milk and somatic cells are maintained totally under control. It is advisable to check the subject at least once a month and, if possible, place it in an intermediate group of milking, that is between negative and positive.

The farmers have joined in a very supportive way in this trial, having no other choice but to reform cure the animal, and were satisfied with the results obtained. As for the subdivision in box, it is necessary to pay attention to the group of negatives and therefore, it should once again take advantage of the potential of screening on bulk milk, milking all the real negative cows or those assumed to be so, and then performing a sample from the tank. This control shall be performed on a weekly basis for the first period, then moved to a monthly scheduling, established on the basis of the incidence of the infection. A focus should also be placed on the environment, but the priority remains the management of the pathogen, to be considered as any other infectious agent.

THE RESULTS

As regards the results obtained, a certain speed of detection and resolution in all herds with low percentages of infection (<5%), where the control of the milk tank was already negative following the elimination of the few infected cows or, at most, within a few months. Conversely, in the stables with many positive bovines, it is necessary to take all possible systems to stop the contagion, as mentioned earlier, in addition to require reasonable hygienic conditions in sheds and especially in the milking room.

In the controls that Agrilab made in the 51 positive herds there was a higher frequency of infections in the post-partum period, with cows that have calved often with already high somatic cell and clinical mastitis. It was also found a very high number of infections after the dry period, therefore, it was investigated the cause. It is assumed that the risk was high due to poor disinfection of the nipple at the time of intramammary antibiotic administration. In fact, even in Canada surveys were carried out on the risk of using injectable drugs directly into the udder. Positive heifer have never been isolated (except the barn where all the animals were positive), but in 68% of cases the infection appeared at the beginning of the second lactation (see Table 1).

Table 1 - Enumeration of somatic cells in a herds with a high percentage of positive cows for Prototheca

GrLat	%	Conta	MdLatte	MdCCS	MdLpicc
1	36	91	25	112	35
2	33	83	28	317	43
3	31	79	28	584	44
Tot	100	253	27	328	40

The group of primiparous cows is the most virtuous in terms of somatic cells.

Increasing lactations gets worse gradually.

GrLat: groups according to lactation;

%: percentage of animals per lactation; Count: number of animals in groups of lactation;

MdLatte: average milk production;

MdCCS: average somatic cell

MdLpicc: average peak milk.

The control of mastitis caused by Prototheca is crucial in order to avoid losses in terms of milk quality and decrease in production due to an increase in somatic cell count, total bacterial load and

to the important environmental elimination of algae. A significant factor is the saving of antibiotics, because if Prototheca was not identified the cows would be treated whenever mastitis appears. Costs have been estimated in order of thousands of Euros, for therapies completely useless, not dictated by a previous and accurate diagnosis and characterized by the paradox that the antibiotic was going to act on the bacteria present in the udder, thus leaving more leeway for the activity of the alga, which in turn can worsen the mastitis symptoms.

In the herds in which it was isolated, Prototheca has always played a major role in influencing somatic cells (see Table 2).

Table 2 - Effect on bulk milk of positive cows for Prototheca

Id	Milk	CCS (x 1.000)	% massa
632	34	2.601	8,3
707	22	3.226	6,7
601	36	1.725	5,8
627	24	1.903	4,3
761	28	1.623	4,3
758	26	1.664	4,1
668	17	2.131	3,4
676	25	1.430	3,4
747	33	1.077	3,3
710	35	923	3,0
695	33	929	2,9
738	23	1.145	2,5
495	22	988	2,0
636	20	1.022	1,9
704	34	587	1,9
732	39	481	1,8
604	16	1.167	1,8
567	22	833	1,7
765	38	479	1,7
696	22	703	1,5
613	36	417	1,4
615	14	1.055	1,4
410	18	804	1,4
764	30	460	1,3

Cows that impact most on bulk milk are highlighted. Among the first 24 bovines of the herds in question, 20 were positive for Prototheca.

Id: identification of the cow;

Milk: production;

CCS: somatic cell counts;

% mass: impact on bulk milk.

Even at the level of individual cows somatic cells are always very high and in direct proportion to the number of colony forming units / ml (see Table 3).

Table 3 - Comparison between somatic cells and colony-forming units / ml in cows found positive

UFC	CSS
210	1.046.000
240	813.000
260	450.000
340	2.085.000
1.500	799.000
2.040	3.906.000
2.180	965.000
2.640	270.000
3.000	623.000
3.760	893.000
4.640	244.000
4.800	275.000
6.000	1.654.000
6.120	2.861.000
12.000	1.976.000
13.000	1.773.000
16.000	1.276.000
17.000	1.450.000
20.000	2.515.000
20.000	1.380.000
20.000	1.957.000
22.000	9.011.000
25.000	1.518.000
30.000	810.000
40.000	1.753.000
40.000	3.521.000
45.000	1.354.000
45.000	1.499.000
50.000	373.000
55.000	3.236.000
80.000	2.915.000
800.000	4.408.000

CFU: colony forming units; CSS: somatic cell counts.

THE RISKS TO THE PURCHASE

Finally, it should be emphasized the risk that occurs upon purchase of a cow from an external company. Agrilab signals a significant number of animals arriving in the Piedmontese farms positive for coagulase + and, less markedly, for *Streptococcus agalactiae* (but with sometimes devastating impacts) and also report positive cases for *Prototheca*. Very often the breeder does not require a certificate attesting the negativity of the bovine, or merely takes in consideration the outcome of somatic cell count, which all too often, however, masks other dangerous pathogens.

CONCLUSIONS

Agrilab , from 2011 to the present, has embarked on a challenging survey of about 25% of the dairy herds of Piedmont , documenting and studying every detail concerning the Prototheca mastitis. The study described has demonstrated that the pathology is underestimated , both in terms of numbers and severity. This evidence is witnessed not only by the number of positive herds , but also from that of the herds that have battled for years against mastitis and continual increases of cells, without ever finding (and now , it can be said " investigating ") the true origin . Very interesting is the figure concerning the type of breeding that sees very large farms, with more than 150-200 cows in lactation, positive for Prototheca .

The presence of the alga has always been associated with small stalls, closed in, with very high humidity, but this survey found a high percentage of positivity in the more modern stables and with a careful hygienic management. A second analysis could lead to a second hypothesis: the small stables have an exchange of animals with the outside much lower than the large ones and , therefore, the latter are subject to greater risk as they introduce veal and neglect the safest system of internal comeback .

The management of a few infected animals provides immediate results, while in cases of higher percentages it is necessary to establish a precise strategy that does not differ from that adopted for any other contagious mastitis agent, at box level and especially in the milking room. The level of attention to hygiene standards must be very high, especially in the phase of administration of intramammary antibiotics. Too often, in fact, these are administered without a careful cleaning of the tip of the nipple , with the consequent introduction of contaminated faeces from Prototheca within the channel . After the adoption of this measure, tangible improvements in the herds infection were observed. The presence of the alga in the faeces of newborn calves justifies the advice to farmers to avoid the administration of infected milk, at least to limit the shedding of the pathogen into the environment.

The number of herds positive for Prototheca in Piedmont is not low and compares to the rates published by the IZS of Lombardy and Emilia Romagna (Piacenza Section -1) and the number of positive herds is higher than those in which there are bovines positive for Streptococcus agalactiae . In consideration of these experiences, every professional should consider this aspect in the range of differential diagnoses of mastitis and a modern professionals belonging to the veterinary category can not disregard the responsible use of antibiotics, for the implications associated with them and for the uselessness of these drugs in this case.

The control and management of the stables followed is still in progress, because prevention is the fulcrum on which rotates the fight against Prototheca .